

Special lecture

Occurrence of Polyacetylenes—Chemistry and Biosynthesis

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Polyacetylene類의 生成—化學 및 生合成

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First, I should like to thank the Korean Society for Pharmacognosy for the kind invitation to give a talk about some of my work. My trip to Seoul was made possible by a travel grant from the Deutsche Forschungsgemeinschaft.

I am sorry that I can not address you in your own language, but I hope we will be able to understand one another in English, with the help of a few slides.

As you will all know among the substances isolated from plants, particularly drug plants, those with one or more acetylene bonds were for a long time a rarity.

Until 1941 five naturally-occurring substances with an acetylene bond were known: Taririnic acid, isonic acid (also known as erythogenic acid), carlinaoxid, lachnophyllum ester, and matricaria ester. Compounds with a number of acetylene bonds were long considered to be accessible only by chemical synthesis. Already Adolf von Bayer concerned himself with other preparation of these compounds. Among the things, he investigated the preparation of diacetylenedicarboxylic acid and tetraacetylene dicarboxylic acid. These compounds proved to be very unstable—they polymerized easily. It was because of this that polyacetylenes were long regarded as compounds which were difficult to synthesize and which were unstable.

The work of W. Reppe (BASF) in the 1930s brought acetylene chemistry once again to the attention of organic chemists. Simple acetylenes were readily available in large quantities, and conditions had improved for the synthesis and study of the properties of polyacetylenes. These investigations were carried out by many different groups. Mentionable are those of Raphael Bergman, Heilbron, Schodkiewiz, Jones, Bohlmann, and Schlubach. The results of these investigations were important for the determination of the structures of multiply-unsaturated compounds, which had been found in micro-organisms. The search for antibiotic substances had revealed some strongly-unsaturated compounds: carboxylic acids, ester, or amides, with one or more triple bonds and one to four ethylene bonds; compounds such as mycomycin, nemotinic acid, agrocybin and diatretin.

At about the same time, more unsaturated compounds of this sort were isolated from higher plants. Since then, over 700 acetylene compounds have been isolated from about 1000 species, which belong to 19 families of plants. The compounds have largely been found in the Compositae and Umbelliferaeae. However, only a few species of the other plant families have been investigated.

These unsaturated compounds also occur in

Higher Plants from which Polyacetylenes were isolated

Family	Approx. Number of Species	Number of Drug Plants isolated
Sterculiaceae	2	
Compositae	ca. 1100	ca. 50
Sapindaceae	1	
Lobeliaceae	1	
Pittosporaceae	1	
Valerianaceae	1	1
Campanulaceae	22	
Annonaceae	1	
Myoporaceae	1	
Opiliaceae	1	
Umbelliferae	195	15
Lauraceae	2	
Araliaceae	7	2
Oliniaceae	1	
Euphorbiaceae	1	
Simarubaceae	4	1
Papilionaceae	3	
Loranthaceae	2	
Santalaceae	19	2
Olacaceae	5	2
Gramineae	2	1
Cupressaceae	1	

cultures of some micro-organisms, particularly basidiomycetes-polyacetylenes have been identified in about 50 species.

Lower Plants from which Polyacetylenes were Isolated

Family	Approximate Number of Species	Number of Drug Plants Included
<i>Telephoraceae</i>	1	
<i>Meruliaceae</i>	1	
<i>Polyporaceae</i>	17	1
<i>Cartiaceae</i>	1	
<i>Cryptococcaceae</i>	2	
<i>Caprinaceae</i>	1	
<i>Tricholomaceae</i>	3	
<i>Strophariaceae</i>	1	
<i>Sreptomycetaceae</i>	1	
<i>Actinomycetaceae</i>	1	

It would appear that these unsaturated substances are widely distributed and that their occurrence is not restricted to a few plant families. But this also poses the question of the significance of these substances for the plant cell-their position in the intermediary metabolism of the cell, and their relationship to the primary and secondary metabolites in the plant.

These naturally-occurring compounds have been isolated only recently not only because the properties of these compounds were for long unknown, but also because these substances are present in only small concentrations and occur together with many closely-related compounds. During the work-up of plant material it is usual to obtain a mixture of polyacetylenes. The separation of individual compounds is a more difficult problem than the structural determination of the individual compounds.

The isolation of the polyacetylenes requires mild conditions since these compounds polymerize readily and some of them are difficult to crystallize. The mixture of compounds is therefore kept in solution and separated by column or layer chromatography or by counter-current distribution. Sometimes chemical treatment is necessary before the components of a mixture can be isolated. Reactions such as manganese dioxide oxidation, sodium borohydride reduction or saponification are commonly used.

Most polyacetylenes are lipophilic-the lipophilicity decreases with decreasing chain length and the accumulation of hydrophilic groups. Very hydrophilic are those polyacetylenes that occur as glycosides. The amino-alkenic acids are also very water soluble. Polyacetylenes can usually be extracted at room temperature from air-dried plant material with petroleum spirit and ether. Light must be excluded. Most polyacetylenes can be detected with high sensitivity and specificity by their UV absorption spectra.

The presence of these substances, even in crude extracts, can be determined from a UV spectrum. Small amounts of polyacetylenes in an extract can also be identified by using high pressure liquid chromatography with a UV spectrometer detector—a UV spectrum is made of each fraction.

The typical curve and high absorptivity of the UV spectra of polyacetylenes are not only of help in detecting these substances, but are also of great value in the determination of their structures.

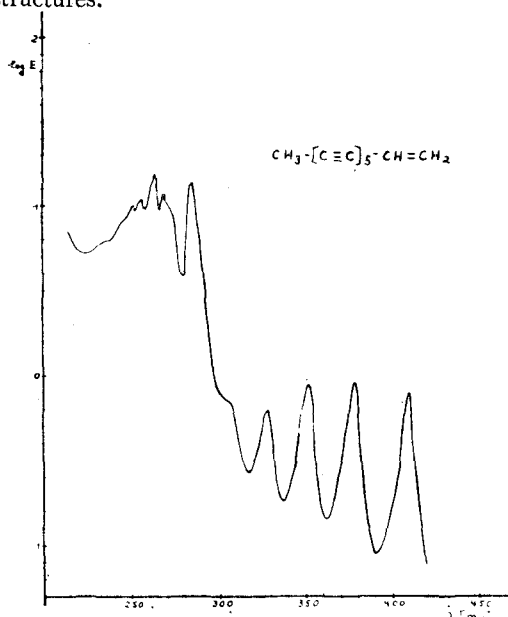


Fig. 4. UV-spectra of tridecen(1)pentain in petroleum isolated from *Arnica montana*.

Compounds with three or more conjugated acetylene bonds have two absorption maxima of very different intensity. They show a characteristic fine-structure, and are usually regarded as two groups of bands. The absorption maxima at short wavelengths can have molar extinctions of over 200,000. This maximum lies above 200nm for substances with three conjugated triple bonds, that is, within the range of the usual UV spectrophotometers. An increase in the number of acetylene bonds in the chromo-

phore system results in a shift to lower wavelengths of both groups of bands. The bands of low intensity are then in the visible region of the spectrum—polyacetylenes with five or more triple bonds are coloured.

The polyacetylenes found in nature often have one or more ethylene bonds, in addition to the acetylene bonds, in the chromophore system. The UV spectra of these poly-yn-enes are characterized by a number of distinct maxima of varying intensity, which can usually be divided into two groups of bands. The difference in the intensity of both groups of bands decreases with a decrease in the number of double bonds. If the number of double bonds is larger than the number of triple bonds, then the spectrum is that of a polyene.

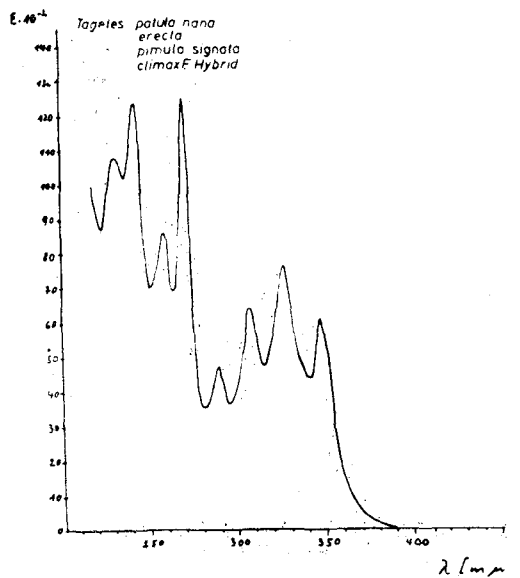


Fig. 5. UV-spectra of Tetradeca-dien(4,6)-triin(8,10,12)-diol(1,3) in methanol isolated from *Tagetes*.

Some rules have emerged from the many spectra of polyynes, polyynenes, and polyenes which have been determined. These rules are valuable for structural determination.

For instance, polyynes and polyynenes can be differentiated from polyenes by measuring the

difference in the frequency of two adjoining absorption bands. The frequency difference for polyynes and polyynes is about 2000cm^{-1} and for polyenes it is about $1,500\text{cm}^{-1}$. The values correspond to the activation energies of the valence electrons of the carbon-carbon triple bond and double bond, respectively.

It is known that the infrared bands for ethylene bonds lie at about $1,600\text{cm}^{-1}$ and those for acetylene bonds at about 2000 to 2100cm^{-1} .

Wave number difference

$$\Delta\nu = \nu_2 - \nu_1$$

$$= \frac{1}{\lambda_2} - \frac{1}{\lambda_1}$$

$-(\text{C}=\text{C})_x$ ca 1500cm^{-1}
 $-(\text{C}\equiv\text{C})_x$ ca 2000cm^{-1}

The frequency difference for a mono-yn-ene chromophore lies between 1500 and 2000cm^{-1} , but one di-yn group in a long polyene chain results in a frequency difference of 2000cm^{-1} .

A further rule for the interpretation of the UV spectra of an unknown substance comes from the change in the spectrum from changes in the proportion of triple bonds to double bonds, The quotient of the highest extinction of the main maximum and the absorption band with the longest wavelength approaches one with an increase in the number of double bonds.

$$\Delta \log E_{(k,l)} = \log E_{(k)} - \log E_{(l)}$$




En : In	$\Delta \log E_{(k,l)}$	UV Region In Which The Most Long-waved UV-Maximum May Be Located (nm)	Number of Measurements	Exceptions
0:3-5	3,9-2,4	513-304	29	4
1:5	1,5-1,4	410	1	-
1:4	1,3-0,9	509-372	3	-
1:3	1,6-0,9	450-325	28	3
1:2	0,5-0,1	295-279	23	3
1:1	0,5-0	374-332	36	-
2:5	1,3	433	1	-
2:4	1,3-1,1	397-382	8	-
2:3	0,7-0,4	375-332	40	1
2:1	0,2-0	367-337	8	-
3:4	0,8	402	1	-
3:2	0,3-0	365-303	47	5

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That is, the difference of the logarithm of these two extinctions decreases with an increase in the number of ethylene bonds, and approaches the value zero. With polyynes it has values of 2.4 to 3.9. With the help of this difference in groups of bonds, it is possible to detect a polyene or polyene chromophore in an unknown substance, with a fair degree of certainty. Further identification of the chromophore can follow from chemical treatment. Partial hydrolysis to a polyene or treatment of a diene group with maleic anhydride are possibilities. The maleic anhydride treatment results in the loss of two double bonds from the chromophore—this results in a shift to the blue.

Infrared spectra are a valuable supplement to electronic spectra for the structural determina-

Characteristic Bands in the Infrared Spectra of Polyacetylene Compounds

Grouping	Band (cm^{-1})
$-\text{C}\equiv\text{CH}$	3300-3240(m), 2100(w-s)
$-(\text{C}\equiv\text{C})_n-$	2260-2100(w-s)
$\text{H}_3\text{C}-\text{C}\equiv\text{C}-$	1380-1375(m)
$\text{H}_3\text{C}-\text{CH}=\text{CH}-$	1370-1365(m)
$-\text{CH}=\text{CH}-$ cis	1660(m), 730-675(m)
$-\text{CH}=\text{CH}-$ trans	1675(m), 965-945(s)
$-(\text{CH}=\text{CH})_2-$ trans	1650-1580(m), 990-980(s)
$-\text{CH}_2-\text{CH}=\text{CH}_2$	3090-3050(w), 1840-1820(w), 1630(w), 1415(m), 990(s), 910(s)
$-\text{C}\equiv\text{C}-\text{CH}=\text{CH}_2$	3040, 1860, 970, 940
$-\text{C}\equiv\text{C}-\text{CH}=\text{CH}-\text{CH}=\text{CH}_2$ trans	990(s), 945(s), 910(s)
$-\text{C}\equiv\text{C}-\text{CO}-\text{CH}=\text{CH}_2$	980(s), 965(s)
$>\text{C}=\text{O}$	1715(s)
$-\text{C}\equiv\text{CH}-\text{CO}-$	1675(m), (1665-1685m)
$-\text{CH}=\text{CH}-\text{CH}=\text{CH}-\text{CO}-$	1665(m)
$-\text{CH}=\text{CH}-\text{CO}-\text{O}-\text{R}$	1740-1735(s), 1210(m)
$-\text{CH}=\text{CH}-\text{CH}_2-\text{O}-\text{CO}-\text{CH}_3$	1775-1740(s), 1240(m)
$-\text{CHO}$	1740-1725(s), 2740(w)
$-\text{OH}$	3650-3400(s-w)
$-\text{C}\equiv\text{C}-\text{CH}=\text{CH}-\text{CHO}$	trans 1682-1688, cis 1677
$-\text{CH}=\text{CH}-\text{S}-\text{CH}_3$	1570-1540
$-\text{HC}-\text{CH}-$ 	1250(s), 950-810, 840-750(s)
	1600-1580(m), 1500-1450(m), 770-730(s), 710-690(ss)
	3140-3080(m), 870-850(s)

tion of polyacetylenes. Infrared spectra are especially useful for detecting functional groups, but are also used to differentiate the unsaturated systems.

I have collected some of the characteristic bands of groups which frequently occur in polyacetylenes in a slide. A detailed discussion of these bands would be out of place at this time.

Characteristic NMR Spectra Signals of Polyacetylenes




Grouping	Chemical Shifting δ (ppm)		
	CH ₃ -Protons	CH ₂ -Protons	CH-Protons
-C≡CH	-	-	1.8-2.5
-CH ₂ -CH ₃	0.9-1.01	1.5-1.6	-
-CH=CH-CH ₃ A B	1.80-1.96	-	A 5.4-5.5 B 6.1-6.4
-C≡C-CH ₃	1.97-2.20	-	-
(-CH ₂) _n -CH=C ^H _A -C ^H _B	-	B 4.8-4.88 C 4.9-5.1	A 5.5-5.74
-CH=CH-CH=C ^H _A -C ^H _B	-	B 5.27-5.40 C 5.36-5.64	A 6.85-6.90
(-C≡C) _n -CH=C ^H _A -C ^H _B	-	B 5.8-6.5 C 5.5-5.6	A 6.0-6.15
-C≡C-CH=CH-	-	-	5.3-6.9
-(CH=CH) ₂	-	-	5.9-7.0
-CH=CH-	-	-	5.4-5.6
(-C≡C) _n -CH=CH-CH ₂ -	-	1.9-2.5	-
-CH ₂ -CHO	-	2.3-2.4	9.68-9.88
-CH=CH-CHO	-	-	tr 3.5-9.6 cis 9.88
-C≡C-CHO	-	-	9.34
-C≡C-CH ₂ -CH=CH-	-	3.0-3.13	-
-C≡C-CH ₂ -OH	-	4.30	-
-CH=CH-CH ₂ -OH	-	4.17	-

Nuclear magnetic resonance spectroscopy has become of great importance in the structural determination of polyacetylenes. The information obtained from UV and IR spectra is not enough to determine the structure of a naturally-occurring polyacetylene. Chemical methods have had to be used—such as perhydration and degradation reactions. Often there was not enough material available for these reactions. The use of NMR spectroscopy together with UV and

IR spectroscopy often allows the complete structure of a polyacetylene to be determined—even if only small amounts of it are available.

Some typical NMR signals are shown in the slide.

Characteristic NMR Spectra Signals of Polyacetylenes

Grouping	Chemical Shifting δ (ppm)		
	CH ₃ -Protons	CH ₂ -Protons	CH-Protons
-CH=CH-CH ₂ -O-CO-CH ₃	1.9-2.06	4.1-4.95	-
-C≡C-CH=CH-CO-O-CH ₃ A B	3.66-3.81	-	A 6.4-6.9 B 6.1-6.4
-CH ₂ -CO-CH ₃	2.01-2.5	2.31	-
-CO-CH ₂ -CH ₂ -CH ₃ A B	0.9	A 2.3-2.4 B 1.5-1.6	-
-S-CH ₃	2.2-2.5	-	-
-SO ₂ -CH ₃	2.96-3.10	-	-
-C≡C-CH ^A -CH ^B -CH ₂	-	B 2.92	A 3.40
-CH=CH-CH ^A -CH ^B -CH ₂	-	-	A 3.04 B 2.74
 -C≡C-	-	-	7.1-7.5
 -C≡C-	-	-	7.1-7.8
-C≡C-  -C≡C-	-	-	6.9-7.1

Mass spectrometry has also been used for the structural determination of polyacetylenes—but it is only of minor importance. Obtaining spectra is difficult since most compounds are unstable, and thermal degradation occurs. If it is possible to volatilize the substance then at least the molecular weight can be determined. With a double-focusing instrument the molecular formula can also be obtained.

Rotatory dispersion can also provide evidence for the structural determination of particular polyacetylenes, as is the case for many other naturally-occurring substances. I shall not go into the details. As well as the spectroscopic methods, chemical methods are also available for the structural determination of polyacetylenes.

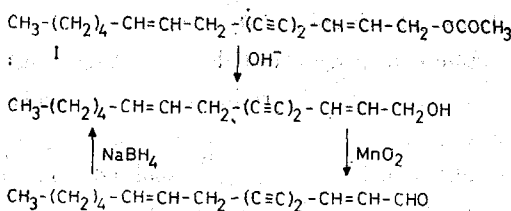
I will not discuss these methods as they are not specific for polyacetylenes, but are used for the structural determination of other naturally-occurring substances.

The isolation and purification of sufficient quantities of a polyacetylene are usually more time-consuming than the determination of its structure. This is made especially difficult by the presence of closely related compounds.

As an example, I should like to present the fourteen unsaturated substances which were recently isolated by us from the umbelliferae *Hydrocotyla asiatica*

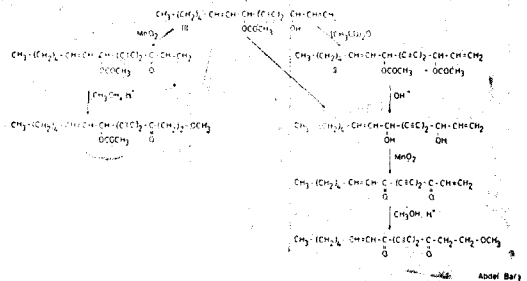
Compound	Polyacetylenes of <i>Hydrocotyla asiatica</i> Constitution and Partialstructure	mg %
I	$\text{CH}_3(\text{CH}_2)_4-\text{CH}=\text{CH}-\text{CH}_2-(\text{C}\equiv\text{C})_2-\text{CH}=\text{CH}-\text{CH}_2-\text{OCOCH}_3$	0.49
II	$\text{CH}_3(\text{CH}_2)_4-\text{CH}=\text{CH}-\text{CH}-(\text{C}\equiv\text{C})_2-\text{CH}=\text{CH}-\text{CH}_2$ $\begin{matrix} \text{OCOCH}_3 & \text{OCOCH}_3 \\ & \\ \text{CH} & \text{CH} \end{matrix}$	2.07
III	$\text{CH}_3(\text{CH}_2)_4-\text{CH}=\text{CH}-\text{CH}-(\text{C}\equiv\text{C})_2-\text{CH}=\text{CH}-\text{CH}_2$ $\begin{matrix} \text{OCOCH}_3 & \text{OH} \\ & \\ \text{CH} & \text{CH} \end{matrix}$	23.01
IV	$\text{CH}_3(\text{CH}_2)_4-\text{CH}=\text{CH}-\text{CH}-(\text{C}\equiv\text{C})_2-\text{CH}=\text{CH}-\text{CH}_2$ $\begin{matrix} \text{OCOCH}_3 & \text{OH} \\ & \\ \text{CH} & \text{CH} \end{matrix}$	11.77
V	$\text{CH}_3(\text{CH}_2)_4-\text{CH}=\text{CH}-\text{CH}-(\text{C}\equiv\text{C})_2-\text{CH}=\text{CH}-\text{CH}_2$ $\begin{matrix} \text{OH} & \text{OH} \\ & \\ \text{CH} & \text{CH} \end{matrix}$	1.88
VI	$\text{CH}_3(\text{CH}_2)_4-\text{CH}=\text{CH}-\text{CH}-(\text{C}\equiv\text{C})_2-\text{CH}_2-\text{OCOCH}_3$ $\begin{matrix} \text{OH} & \text{OH} \\ & \\ \text{CH} & \text{CH} \end{matrix}$ $(n, m = 6)$	0.26
VII	$\text{R}^1-\text{CH}=\text{CH}-(\text{C}\equiv\text{C})_2-\text{CH}=\text{CH}-\text{CH}-\text{R}^2$ $\begin{matrix} \text{OCOCH}_3 \\ \\ \text{CH} \end{matrix}$	0.07
VIII	$-(\text{C}\equiv\text{C})_2-\text{CH}=\text{CH}-\text{CH}_2-\text{OCOCH}_3-\text{OH}$	0.05
IX	$-(\text{C}\equiv\text{C})_2-\text{CH}=\text{CH}-\text{CH}_2-\text{OCOCH}_3-\text{OH}$	0.02
X	$-\text{CH}=\text{CH}-(\text{C}\equiv\text{C})_2-\text{CH}=\text{CH}-\text{CH}_2-\text{OCOCH}_3-\text{OH}$	0.03
XI	$-(\text{C}\equiv\text{C})_2-\text{CH}=\text{CH}-\text{CH}_2-\text{OCOCH}_3-\text{OH}$	0.07
XII	$\text{R}^1-\text{CH}=\text{CH}-(\text{C}\equiv\text{C})_2-\text{CH}=\text{CH}-\text{CH}_2-\text{OH}$	0.07
XIII	$-\text{CH}=\text{CH}-(\text{C}\equiv\text{C})_2-\text{CH}=\text{CH}-\text{CH}_2-\text{OH}$ $\begin{matrix} \text{OH} \\ \\ \text{CH} \end{matrix}$	0.04
XIV	$-(\text{C}\equiv\text{C})_2-\text{CH}=\text{CH}-\text{CH}_2-\text{OH}$ $\begin{matrix} \text{OH} \\ \\ \text{CH} \end{matrix}$	0.03

The polyacetylenes one (I) to four (IV) not only have the same number of carbon atoms, they also have similarities in their unsaturated systems and in their functional groups.



Abdel Bary

Compound one is a fifteen carbon alkynol acetate which was contaminated with a carbonyl compound, which could not be removed by chromatography. The ketone was detected by infrared spectroscopy and by reduction with sodium borohydride. The separation was finally successful with the Girard-T-reagent via the water-soluble hydrazone. The structure of the pure compound was established from spectral data and some chemical reactions. Mild hydrolysis of the ester with 0.5% methanolic KOH gave the unsaturated alcohol, which could be oxidized to the aldehyde with activated MnO_2 , and the aldehyde could be reduced back to the alcohol.



Compound three could also not be purified by chromatography. Infrared spectra showed an OH group. Treatment with acetic anhydride gave the ester which could be separated from the accompanying substances. Saponification of the acetate gave an alkyndiol. Manganese dioxide oxidation gave an alkyndione, the vinyl group of which reacted quickly with acidic methanol to form the 1-methoxyalkyndione. Finally it could be shown that the substance was a pentadecadien-diyndiol monoacetate.

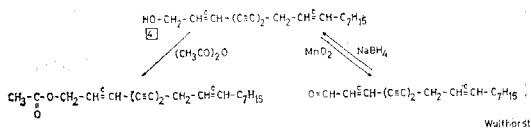
From the umbelliferae *Aegopodium podagraria* we have been able to separate 13 polyacetylenes with the same or a similar chromophore system. Eleven of them are seventeen-carbon alkyn-en-ols, in some of which there is

Polyacetylenes of *Aegopodium podagraria*

- [1] $\text{CH}_2=\text{CH}-\underset{\text{OH}}{\underset{|}{\text{C}}}-(\text{C}\equiv\text{C})_2-\text{CH}_2-\text{CH}=\underset{\text{OH}}{\underset{|}{\text{C}}}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_3$
- [2] $\text{CH}_2=\text{CH}-\underset{\text{O}}{\underset{||}{\text{C}}}-\text{C}(\text{C}\equiv\text{C})_2-\text{CH}_2-\text{CH}=\underset{\text{OH}}{\underset{|}{\text{C}}}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_3$
- [3] $\text{HO}-\text{CH}_2-\text{CH}=\underset{\text{OH}}{\underset{|}{\text{C}}}-\text{C}(\text{C}\equiv\text{C})_2-\text{CH}_2-\text{CH}=\underset{\text{OH}}{\underset{|}{\text{C}}}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_3$
- [4] $\text{HO}-\text{CH}_2-\text{CH}=\underset{\text{OH}}{\underset{|}{\text{C}}}-\text{C}(\text{C}\equiv\text{C})_2-\text{CH}_2-\text{CH}=\underset{\text{OH}}{\underset{|}{\text{C}}}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_3$
- [5] $\text{CH}_2=\text{CH}-\underset{\text{OH}}{\underset{|}{\text{C}}}-\text{C}(\text{C}\equiv\text{C})_2-\underset{\text{O}}{\underset{||}{\text{C}}}-\text{CH}=\underset{\text{OH}}{\underset{|}{\text{C}}}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_3$
- [6] $\text{CH}_2=\text{CH}-\underset{\text{OH}}{\underset{|}{\text{C}}}-\text{C}(\text{C}\equiv\text{C})_2-\underset{\text{O}}{\underset{||}{\text{C}}}-\text{CH}=\underset{\text{OH}}{\underset{|}{\text{C}}}-\text{CH}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_3$
- [7] $\text{CH}_2=\text{CH}-\underset{\text{OH}}{\underset{|}{\text{C}}}-\text{C}(\text{C}\equiv\text{C})_2-\underset{\text{O}}{\underset{||}{\text{C}}}-\text{CH}=\underset{\text{OH}}{\underset{|}{\text{C}}}-\text{CH}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_3$
- [8] $\text{CH}_2=\text{CH}-\underset{\text{O}}{\underset{||}{\text{C}}}-\text{C}(\text{C}\equiv\text{C})_2-\underset{\text{OH}}{\underset{|}{\text{C}}}-\text{CH}=\underset{\text{OH}}{\underset{|}{\text{C}}}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_3$
- [9] $\text{CH}_2=\text{CH}-\underset{\text{OH}}{\underset{|}{\text{C}}}-\text{C}(\text{C}\equiv\text{C})_2-\underset{\text{OH}}{\underset{|}{\text{C}}}-\text{CH}=\underset{\text{OH}}{\underset{|}{\text{C}}}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_3$
- [10] $\text{CH}_2=\text{CH}-\underset{\text{OH}}{\underset{|}{\text{C}}}-\text{C}(\text{C}\equiv\text{C})_2-\underset{\text{OH}}{\underset{|}{\text{C}}}-\text{CH}=\underset{\text{OH}}{\underset{|}{\text{C}}}-\text{CH}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_3$
- [11] $\text{CH}_2=\text{CH}-\underset{\text{OH}}{\underset{|}{\text{C}}}-\text{C}(\text{C}\equiv\text{C})_2-\underset{\text{OH}}{\underset{|}{\text{C}}}-\text{CH}=\underset{\text{OH}}{\underset{|}{\text{C}}}-\text{CH}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_3$
- [12] $\text{CH}_2=\text{CH}-\underset{\text{OH}}{\underset{|}{\text{C}}}-\text{C}(\text{C}\equiv\text{C})_2-\text{CH}=\underset{\text{OH}}{\underset{|}{\text{C}}}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_3$
- [13] $\text{HO}-\text{CH}_2-\text{CH}=\underset{\text{OH}}{\underset{|}{\text{C}}}-\text{C}(\text{C}\equiv\text{C})_2-\text{CH}=\underset{\text{OH}}{\underset{|}{\text{C}}}-\text{CH}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_3$

Wulfhorst

also a CO group or an acetylated OH group. Among the seventeen-carbon-alkyn-en-ol there are three pairs of isomers which differ only in the configuration of a double bond or of the asymmetric carbon of a secondary OH group.

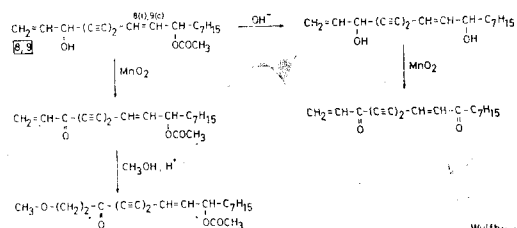


Wulfhorst

The isolation of compound four was particularly difficult since this primary alcohol was contaminated with two other alkynols. Oxidation of the mixture with manganese dioxide enabled chromatographic separation. Two acetyleneals were obtained which differed from one another only in the configuration of the non-conjugated double bond.

The unsaturated aldehydes could not only be characterized by the spectroscopic properties but also by the ready formation of acetals in me-

thanol. The acetal formation could be followed and measured by UV spectroscopy—these is a hypochromic shift of about 30nm. The aldehydes were identified as compounds four and five. The third oxidation product was also an aldehyde, which also readily formed the acetal. The partial structure of an alkendiyne-en-diol could be established, but the molecular weight could not be determined exactly.



Wulfhorst

Compounds eight and nine could be separated by column chromatography but they have the same molecular weight and practically identical UV spectra. They differ from one another by their optical activity: 8 is levorotatory and 9 is dextrorotatory. In the infrared spectrum of 8 there is a band at 952cm^{-1} which is typical for trans double bonds. Compounds eight and nine can be saponified to diols and then oxidized with manganese dioxide to the diketone with an ene-one-diyne-ene-one chromophore. We had already identified polyacetylenes with this chromophore in *Hydrocotyle asiatica*. Compounds 8 and 9 are readily oxidized to a ketone which readily reacts with methanol. This established that the secondary OH group is at carbon-three. We could not establish whether the conformational difference between 8 and 9 is at carbon-three or carbon-ten, or both.

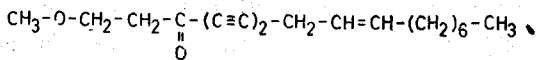
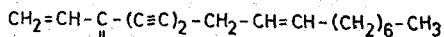
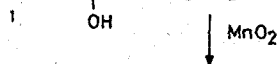
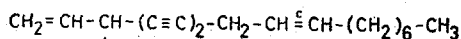
The desert plant *Pityranthus tortuosus*, also an umbelliferae, is used by the bedouins to prepare medicines. We now been able to isolate twelve polyacetylenes from this plant. The compounds are seventeen-carbon alkynenols and alkynenones which have an identical saturated

Polyacetylenes of *Pitufanthus lotuosus*

Compound	Constitution
1	$\text{CH}_2=\text{CH}-\underset{\text{OH}}{\text{CH}}-(\text{C}\equiv\text{C})_2-\text{CH}_2-\text{CH}=\text{CH}-(\text{CH}_2)_6-\text{CH}_3$
2	$\text{CH}_2=\text{CH}-\underset{\text{O}}{\text{C}}-(\text{C}\equiv\text{C})_2-\text{CH}_2-\text{CH}=\text{CH}-(\text{CH}_2)_6-\text{CH}_3$
3	$\text{HO}-\text{CH}_2-\text{CH}=\text{CH}-(\text{C}\equiv\text{C})_2-\text{CH}_2-\text{CH}=\text{CH}-(\text{CH}_2)_6-\text{CH}_3$
4	$\text{CH}_2=\underset{\text{OH}}{\text{CH}}-\text{CH}-(\text{C}\equiv\text{C})_2-\underset{\text{OH}}{\text{CH}}-\text{CH}-(\text{CH}_2)_6-\text{CH}_3$
5	$\text{CH}_2=\underset{\text{OCOCH}_3}{\text{CH}}-\text{CH}-(\text{C}\equiv\text{C})_2-\underset{\text{OH}}{\text{CH}}-\text{CH}-(\text{CH}_2)_6-\text{CH}_3$
6	$\text{CH}_2=\underset{\text{OH}}{\text{CH}}-\text{CH}-(\text{C}\equiv\text{C})_2-\underset{\text{OCOCH}_3}{\text{CH}}-\text{CH}-(\text{CH}_2)_6-\text{CH}_3$
7	$\text{CH}_2=\underset{\text{OH}}{\text{CH}}-\text{CH}-(\text{C}\equiv\text{C})_2-\text{CH}=\underset{\text{OH}}{\text{CH}}-(\text{CH}_2)_6-\text{CH}_3$
8	$\text{CH}_2=\underset{\text{OH}}{\text{CH}}-\text{CH}-(\text{C}\equiv\text{C})_2-\text{CH}=\underset{\text{OH}}{\text{CH}}-(\text{CH}_2)_6-\text{CH}_3$
9	$\text{CH}_2=\underset{\text{OH}}{\text{CH}}-\text{CH}-(\text{C}\equiv\text{C})_2-\underset{\text{OCOCH}_3}{\text{CH}}-\text{CH}-(\text{CH}_2)_6-\text{CH}_3$
10	$\text{CH}_2=\underset{\text{OH}}{\text{CH}}-\text{CH}-(\text{C}\equiv\text{C})_2-\underset{\text{O}}{\text{CH}}-\text{CH}-(\text{CH}_2)_6-\text{CH}_3$
11	$\text{CH}_2=\underset{\text{OH}}{\text{CH}}-\text{CH}-(\text{C}\equiv\text{C})_2-\underset{\text{O}}{\text{CH}}-\text{CH}-(\text{CH}_2)_6-\text{CH}_3$
Partial structure	
12	$\text{R}_1-\underset{\text{O}}{\text{C}}-(\text{C}\equiv\text{C})_2-\text{CH}_2-\text{CH}=\text{CH}-\text{R}_2$

Pötter

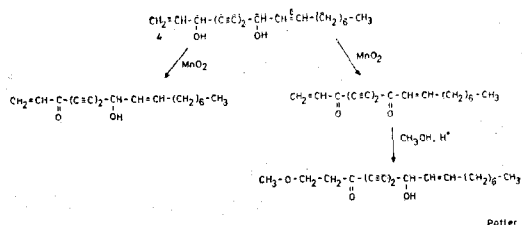
group C_7H_{15} and unsaturated ten-carbon fragments with similar chromophores.



Pötter

Compound one is the alcohol corresponding to compound two. It is known as faltarinol, panaxynol or carotatoxin and has been identified in *Panax ginseng*, *Daucus carota* and some other plants.

Compound four separated with small amounts of compound seven, but they could be chroma-

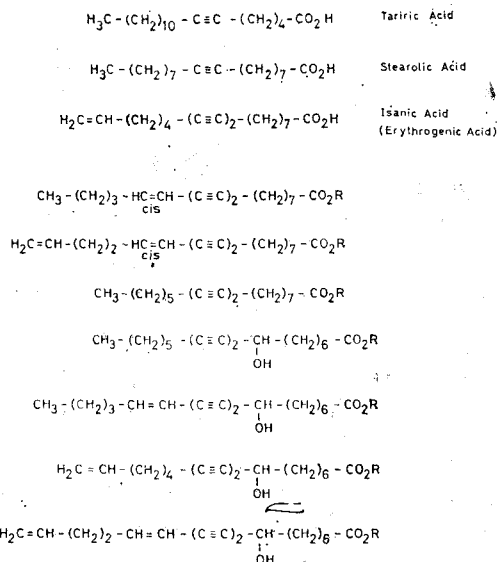


Pötter

tographically separated. Both compounds are optically active diols which differ only in the position and the configuration of the double bond and in the position of the second secondary OH group. Compound four is faltarindiol-oxidation with manganese dioxide produces two compounds: faltarinolone and faltarindione. The spectra of these two compounds shows a shift to red compared with that of compound four (4.56 and 11.5nm). The vinyl group of both compounds reacts readily with methanol.

Compound seven is again an isomer of compound 8, it differs only in the configuration of the double bond. Compounds 7 and 8 are the ketoalcohols corresponding to the dioles 10 and 11. The difference between 10 and 11 is also only the configuration of the double bond.

I do not see much point in attempting to give an idea of the many-sidedness of this class of substances by giving further examples from our group. And it would be too much for me to list the many acetylenes that have been identified, either chronologically or according to their occurrence in particular plants or microorganisms. Rather, I should like to show you some representative examples of these naturally-occurring substances, arranged according to chemical differences, and I should like to comment on some biogenetic relationships. This should help to complete the picture. It will not be possible to name every author who has enriched the chemistry of this class of compounds. Significant advances have been made by the groups of Bohlmann, Sørensen, Jones, and



Bu'Lock.

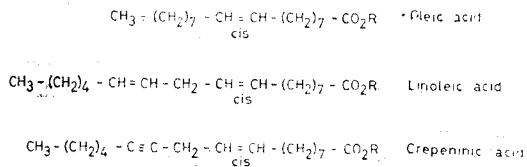
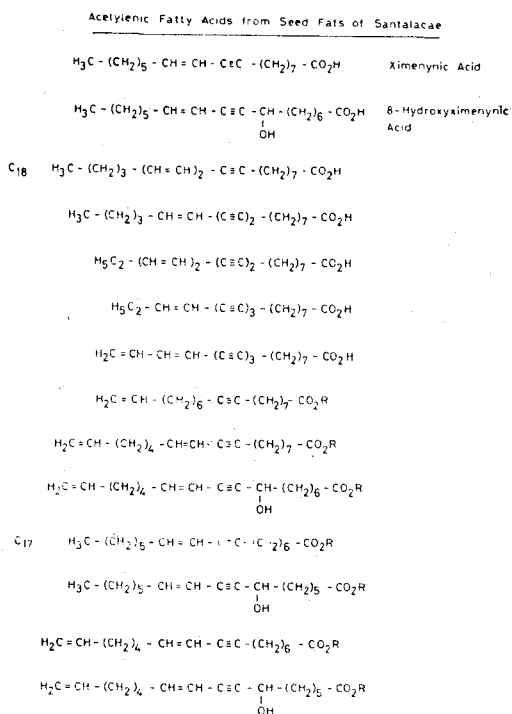
I have already mentioned taririnic acid in the introduction. It is an isomer of stearic acid and occurs in the seed-oil of *Picramnia tariri*. The acid, which is also found in the seed-oil of other *Picramnia* species, was for long an isolated

finding. In 1940 Steger and van Loon isolated isanic acid, an eighteen-carbon ene-diinic acid, from boleko oil, or isan oil, which is obtained from the nuts of the ongokea tree which grows wild in the Congo.

Since then isan oil has been investigated from various aspects. We now know that an additional seven eighteen-carbon alkyenic acids occur in it, and that together they account for 75% of the total fatty acids.

In 1950 Leighelm and co-workers discovered ximeninic acid, accompanied by 8-hydroxyximeninic acid, in the kernel oil of some South African *Ximenia* species.

Other eighteen-carbon acids with 2 to 3 acetylene and 1 to 2 ethylene bonds were identified in the seed-oil and in other parts of closely related species of the santalaceae. Seventeen-carbon alkyenic acids occur in this seed oil alongside the eighteen-carbon alkyenic acids. The mixture of acids was separated, in part, by counter current distribution.

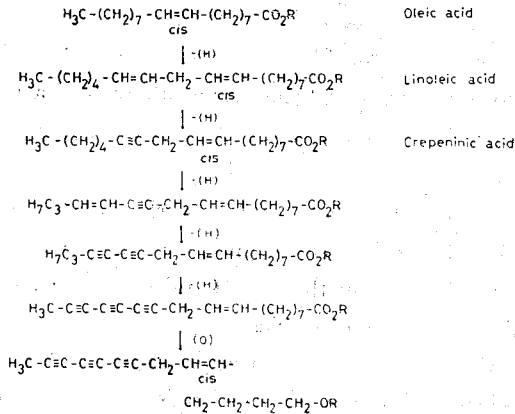


An interesting eighteen-carbon acetylenic acid is crepennic acid, whose cis double bond is at the 9, 10 position, as it is in oleic acid. Crepennic acid was first found in the seed-oil of the compositae *Crepis foetida*.

However, alkyenic acids do not only occur in the triglycerides of seed-oil, they also occur in higher plants and in the culture broth of microorganisms-although in much smaller quantities.

In those substrates the acetylenic acids usually occur as methyl esters, sometimes as amides or nitriles. They are of varying chain length and vary in their degree of saturation. They occur together with other polyacetylenes, which often

have the same or a similar chromophore, and which can be identified as hydrocarbons, alcohols, ketones, aldehydes and epoxides with phenyl, thienyl and furfunyl groups.



That many polyacetylenes have an unbranched carbon chain and often have the same or a similar chromophore leads one to the belief that. Biogenetic relationships exist to the fatty acids, in particular oleic acid and linolic acid. This assumption was supported by the finding that these compounds are built up from acetate and malonate units in the plant cell. That is, their biosynthesis is closely related to that of the fatty acids.

Feeding of labelled oleic acid has shown that micro-organisms and higher plants can not only utilize oleic acid to synthesize eighteen-carbon alkyenic acids and short chain unsaturated acids such as, for example, the ten-carbon dehydromatricariaic acid, but also alcohols, hydrocarbons and spiroketals. Only oleic acid can be used as the precursor-not the isomers of these acids. The biosynthesis most probably runs via linolic acid, crepeninic acid, and dehydrocrepeninic acid from which the eighteen-carbon diyn-ene-acids and the eighteen-carbon triyn-ene-acids are formed. Alpha or beta oxidation gives the short-chain homologues with identical chromophores.

Number of Carbons	Structure	Source	Reference
10	$\text{CH}_3-\text{CH}=\text{CH}-\text{C}\equiv\text{C}-\text{C}\equiv\text{C}-\text{CH}=\text{CH}-\text{COOCH}_3$ cis	Matricaria est. Matricaria inodora	N. A. Sørensen et al 1941
10	$\text{CH}_3-\text{CH}=\text{CH}-\text{C}\equiv\text{C}-\text{C}\equiv\text{C}-\text{COOCH}_3$ cis	Lachnophyllum ester Lachnophyllum gossypium Bellis perennis	W. W. Wilgams W. S. Smirnow W. P. Gollman 1935
10	$\text{CH}_3-\text{CH}=\text{CH}-\text{C}\equiv\text{C}-\text{C}\equiv\text{C}-\text{COOCH}_3$ cis	cis-Dehydromatricaria ester Achillea millefolium	F. Bohmann et al 1952
10	$\text{CH}_3-\text{CH}=\text{CH}-\text{C}\equiv\text{C}-\text{C}\equiv\text{C}-\text{COOCH}_3$ cis	cis-Dihydromatricaria ester Arnica strigosa	N. A. Sørensen et al 1954

Lachnophyllic acid, ten-carbon diyn-enic acid was isolated as the methylester from the ethereal oil of the Compositae Lachnophyllum gossypium by some Russian chemists in 1935. The ethereal oil is used to perfume soaps. This discovery remained largely unknown until 1941 when Sørensen in Drontheim found the methylester of an alkyenic acid in the ethereal oil of an odourless Camille. He identified the acid as ten-carbon ene-diyn-enic acid and named it matricariaic acid. This discovery was made after steam distillation of the plant material in search for the matricaria camphor.

Both acids, lachnophyllic acid and matricariaic acid have since been identified in many other plants. Such as, for example, in Bellis perennis the daisy that decroates our lawns and meadows in the spring. The esters are sometimes accompanied by dihydromatricariaic ester and dehydromatricariaic ester, and occur as cis/cis, trans/trans, cis/trans, and trans/cis isomers.

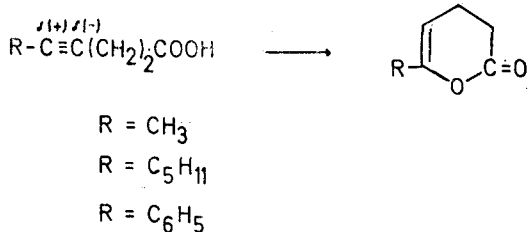
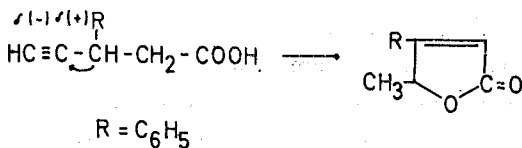
Number of Carbons	Structure
10	$\text{H}_3\text{C}-\text{C}\equiv\text{C}-\text{C}\equiv\text{C}-\text{C}\equiv\text{C}-\text{CH}=\text{CH}-\text{COOCH}_3$ tr
10	$\text{H}_3\text{C}-\text{C}\equiv\text{C}-\text{C}\equiv\text{C}-\text{C}=\text{CH}-\text{CH}=\text{CH}-\text{COOCH}_3$ tr H ₃ C-S
10	$\text{H}_3\text{C}-\text{C}=\text{CH}-\text{C}\equiv\text{C}-\text{C}\equiv\text{C}-\text{CH}=\text{CH}-\text{COOCH}_3$ c tr H ₃ C-S
10	$\text{H}_3\text{C}-\text{C}\equiv\text{C}-\text{C}=\text{CH}-\text{C}\equiv\text{C}-\text{CH}=\text{CH}-\text{COOCH}_3$ tr c H ₃ C-S
10	$\text{H}_3\text{C}-\text{C}\equiv\text{C}-\text{CH}=\text{CH}-\text{C}\equiv\text{C}-\text{CH}=\text{CH}-\text{COOCH}_3$ c tr c H ₃ C-S

Thioenolesters of these ten-carbon alkynenic acids which also occur in plants, may also be biogenetically related to these acids.

Lactones

Number of Carbons	
10	$H_3C-CH\equiv CH-C\equiv C-CH=\overset{O}{\parallel}O$ Matricarialactone
10	$H_3C-CH_2-CH_2-C\equiv C-CH=\overset{O}{\parallel}O$
10	$H_3C-CH\equiv CH-CH=C=C=\overset{O}{\parallel}O$ Lachnophyllum-X-Lactone
10	$H_3C-C\equiv C-C\equiv C-CH=\overset{O}{\parallel}O$
10	$H_3C-C\equiv CH-C\equiv C-CH-\overset{O}{\parallel}O$ CH_3-SO_2
10	$H_3C-C\equiv C-C\equiv CH-CH=\overset{O}{\parallel}O$ CH_3-S
17	$RO-CH_2-\underset{OR}{\underset{ }{CH}}-CH_2-\overset{O}{\parallel}O-(CH_2)_8-C\equiv CH$ R = H, Ac Rubrynilide

Relationships can also be seen between the unsaturated acids and the unsaturated lactones which have been isolated from various plants. Lactone formation can result from nucleophilic addition of the carboxyl group to the triple bond.



Even short-chain alkynoic acids such as 4-pentynoic acid and 4-Hexynoic acid, undergo this reaction, as was demonstrated in my laboratory in 1953. Unsaturated gamma or delta

lactones are found such as angelic lactone or phenyl-cumalin.

Hydrocarbons

Number of Carbons	
13	<i>en-pentayne</i> $CH_2-C\equiv C-C\equiv C-C\equiv C-C\equiv C-CH=CH_2$
12	<i>en-tetrayn-ene</i> $H-CH=CH-C\equiv C-C\equiv C-C\equiv C-CH=CH-H$
13	CH_3
14	CH_3 -CH ₃
13	<i>dien-tetrayne</i> $H_2C=CH-CH=CH-C\equiv C-C\equiv C-C\equiv C-CH_3$
13	<i>dien-triyn-ene</i> $H_2C=CH-CH=CH-C\equiv C-C\equiv C-C\equiv C-CH=CH-CH_3$
13	$H_2C=C$ -CH ₃
	Cl
12	<i>en-triyn-ene</i> $C_2H_5-CH=CH-C\equiv C-C\equiv C-C\equiv C-CH=CH-CH_3$
12	<i>dien-triyn-ene</i> $CH_2=CH-CH=CH-C\equiv C-C\equiv C-C\equiv C-CH_3$
17	$CH_2CH(CH_2)_2$ -CH ₃ ..CENTAUR X ₃
6	<i>triyn-ene</i> $HC\equiv C-C\equiv C-CH$ *
12	<i>dien-diyne-diene</i> $H_2C=CH-CH=CH-C\equiv C-C\equiv C-CH=CH-CH_2$
13	<i>triene-diyne-ene</i> $H_2C=CH-CH=CH-CH=C\equiv C-C\equiv C-CH=CH-CH_3$
17	<i>dien-diyne-ene</i> $CH_2=CH-(CH_2)_2-CH=CH-CH=C\equiv C-C\equiv C-CH=CH-CH_3$
13	CH_3-CH_2 -CH ₃ ..ETHUSIN

Besides esters and lactones, strongly unsaturated hydrocarbons have also been found in higher plants. Some types of these are described in the next slide. Most of those substances occur together with other polyacetylenes. Among them is tridecenpentin which has been identified in small quantities in many higher plants.

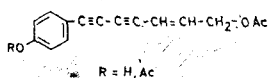
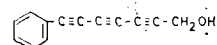
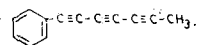
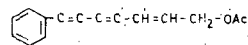
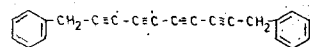
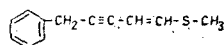
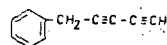
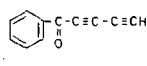
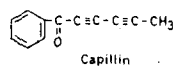
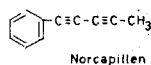
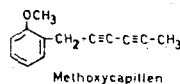
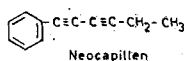
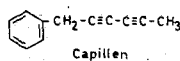
ALCOHOLS

Number of Carbons	
14	<i>dien-diyne-ene</i> $C_3H_7-CH=CH-CH=C\equiv C-C\equiv C-CH=CH-CH_2OH$ Aethusalol B
13	$CH_2(OH)-CH(OH)-CH=CH-CH=C\equiv C-C\equiv C-CH=CH-CH_3$
13	<i>dien-triene</i> $CH_2-C\equiv C-C\equiv C-CH=CH-CH=CH-CH=CH-CH_2-CH_2-OH$ Chamomilla-Alcohol
17	$CH_2=CH-(CH_2)_4-C\equiv C-C\equiv C-CH=CH-CH=CH-CH=CH-CH_2-OH$ <i>en-diyne-ene</i>
12	$CH_2-(CH_2)_2-CH\equiv C-C\equiv C-C\equiv C-CH\equiv C-CH_2-OH$ * <i>trans-Matricarialol</i>
13	$CH_3-CH=CH-C\equiv C-C\equiv C-CH\equiv C-CH(OH)-C_3H_7$ Aethusalol B
	<i>dien-ene</i>
9	$HO-CH_2-CH_2-C\equiv C-C\equiv C-CH=CH-CH_2OH$ *
9	$H-C\equiv C-C\equiv C-CH=CH-(CH_2)_3-OH$
	<i>en-diyne</i>
10	$CH_2=CH=CH-C\equiv C-C\equiv C-(CH_2)_3-OH$
9	$H-CH=CH-C\equiv C-C\equiv C-CH(OH)-CH(OH)-CH_2(OH)$ *
7	$H-C\equiv C-C\equiv C-CH(OH)-CH(OH)-CH_2(OH)$ *
	<i>diyne</i>
9	$CH_3-CH_2-CH(OH)-C\equiv C-C\equiv C-CH(OH)-CH_2(OH)$ *
	<i>dien-diene</i>
9	$HC\equiv C-C\equiv C-CH=C-CH-CH_2-CH_2-OH$ * (+) and (-) Marasine
10	$HC\equiv C-C\equiv C-CH=C-CH-(CH_2)_3-OH$
9	$HC\equiv C-C\equiv C-CH=C-CH-CH(OH)-CH_2-OH$ *
11	$HC\equiv C-C\equiv C-CH=C-CH-(CH_2)_3-CH_2-OH$ *
11	$HC\equiv C-C\equiv C-CH=C-CH-CH(OH)-(CH_2)_2-CH_2-OH$ *
8	$HC\equiv C-C\equiv C-CH=C-CH-CH_2-OH$
11	$HC\equiv C-C\equiv C-CH=C-CH-CH(OH)-CH_2-CH_2-OH$ *
10	$HC\equiv C-C\equiv C-CH=C-CH-CH(OH)-CH_2-CH_2-OH$ *

Aldehydes of various chain lengths and with various chromophores have also been found.

Number of Carbons	Chemical Structure	Source
14	$\text{CH}_3\text{-C}\equiv\text{C-C}\equiv\text{C-C}\equiv\text{C-CH=CH-CH}_2\text{-CH(CH}_3\text{)}_2\text{-OH}$	Pontica-epoxid, Artemisia pontica
13	$\text{CH}_3\text{-C}\equiv\text{C-C}\equiv\text{C-C}\equiv\text{C-CH=CH-CH}_2\text{-CH=CH}_2$	Achilla Millefolium
13	$\text{CH}_3\text{-C}\equiv\text{C-C}\equiv\text{C-C}\equiv\text{C-CH}_2\text{-CH=CH-CH=CH-CH}_2$	Cota-epoxid, Anthemis cota
13	$\text{CH}_3\text{-CH=CH-C}\equiv\text{C-C}\equiv\text{C-CH=CH-CH}_2\text{-CH}_2\text{-CH}_3$	Aethusin-epoxid, Aethusa cynapium
13	$\text{HO-CH}_2\text{-CH=CH-C}\equiv\text{C-C}\equiv\text{C-CH=CH-CH}_2\text{-CH}_2\text{-CH}_3$	Aethusanol-B-epoxid, Aethusa cynapium
13	$\text{CH}_3\text{-C}\equiv\text{C-C}\equiv\text{C-C}\equiv\text{C-CH=CH-CH}_2$	
13	$\text{CH}_3\text{-CH=CH-C}\equiv\text{C-C}\equiv\text{C-C}\equiv\text{C-CH=CH-CH}_2$	
9	$\text{H-C}\equiv\text{C-C}\equiv\text{C-C}\equiv\text{C-CH}_2\text{-CH}_2\text{-OH}$	

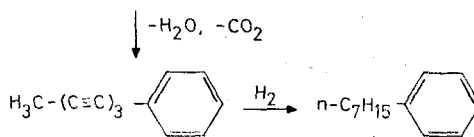
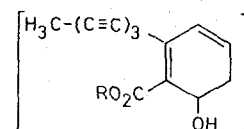
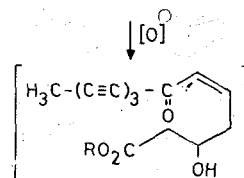
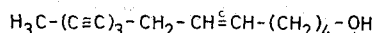
Expoxides such as pontica epoxide and cota epoxide have been known for some time. Many alkyloxiranes are now known and I will not mention any by name.



In 1944 Treibs described a phenyl substitute alkyne derivative it was agropyren, which was later shown to be identical with capillen, which

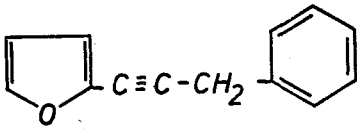
had been isolated from *Artemisia capillaris*, in 1930. Capillen, which forms up to 80% of the etheral oil of *Artemisia capillaris*, is oxidized by air and light to 1-phenyl-2,4-hexadiyn-1-one. Ismai in 1956, isolated this oxidation product and called it capillin. Capillin has strong fungicidal actions, but is too toxic for therapeutic use. Many homologues of agropyren have since been isolated from various plants.

Recently norcapillen, neocapillen and ortho-methoxy-capillin have been isolated from *Artemisia capillaris*. Some of the compounds have additional functional groups such as an OH group or a thienolether group.



The biosynthesis of phenylalkynes most likely proceeds via an aliphatic chain, no preformed aromatic such as benzoic acid or phenylacetic acid are incorporated. Two different pathways for the formation of the phenyl ring have been proposed on the basis of experimental findings. They differ according to the type of substituent.

The investigations of F.W. Semmler on the etheral oil of *Carlina acaulis* were performed more than 75 years ago. Semmler proposed an allene structure for carlina-oxide-this was corrected in 1933: carlina-oxide is 1-phenyl-3-furyl



Carlinaoxyde

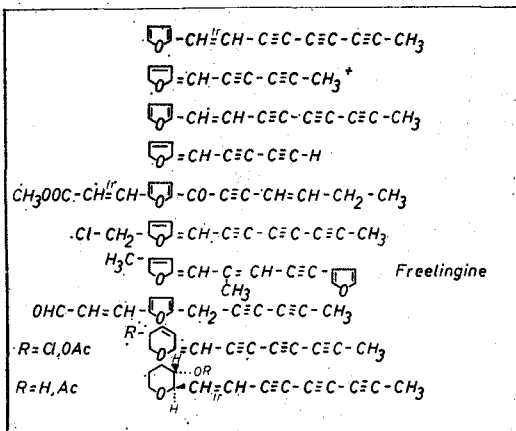
F. W. Semmler 1889 - 1909

A. St. v. Pfau, J. Pictel,

Pl. Platner u. B. Susz 1935

-3-propyne. This compound was synthesized in 1936 by some Swiss chemists.

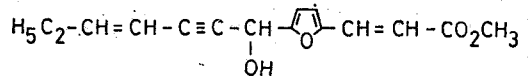
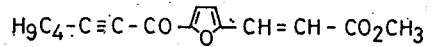
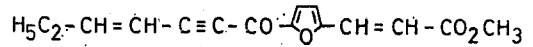
J. Schmidt-Thomé demonstrated that an extract of the roots of *Carlina acaulis* had strong bacteriostatic activity against Gram-positive bacteria. This antibiotic effect was all due to the carlina-oxide. Animal tests should that carlina-oxide was too toxic a property that it shows with many other acetylene compounds.



Other furyl-alkynes have been isolated, most of which have the unsaturated chain at carbon-two. But there are some furan derivatives that have an additional substituent at position 5 or 4. We find the first substance with a methyl-branching among these furan derivatives. It is freelingin which was discovered in 1966.

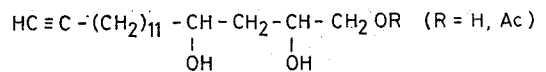
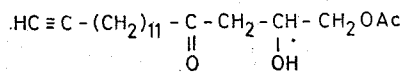
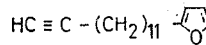
Alkynyl-substituted tetrahydropyrans have also been isolated from various Compositae.

Alkynylfurylacrylates from *Vicia faba*



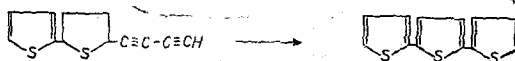
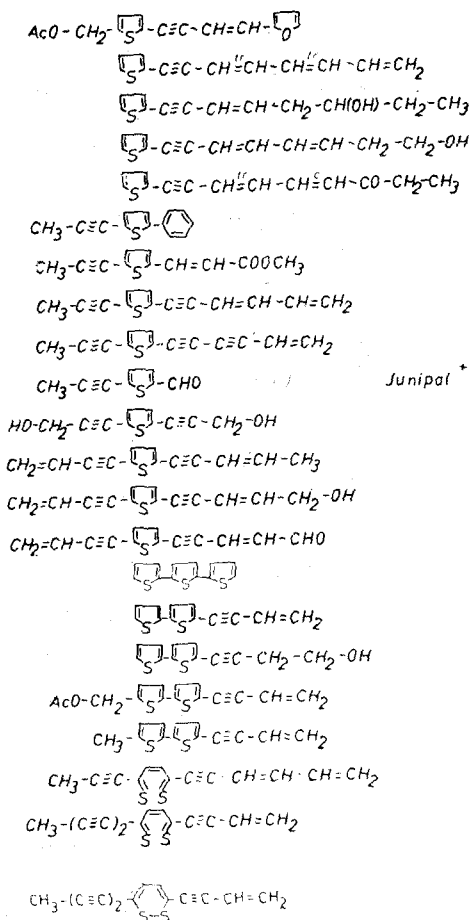
The unsaturated compounds that occur in the field bean also belong to the alkynylfurans. These are wyeron, wyerol, dehydrowyeron, and dehydrowyerol. The free acid of wyeron is identical with phytoalexin, which has fungo-static effects in concentrations of 9 to 45 micrograms per milliliter, and prevents, among other things, the germination of the parastic fungus (*Botrytis fabae*). This fungus causes leaf drop and eventual death of the bean plant.

Persea gratissima (Avocado - Pear)



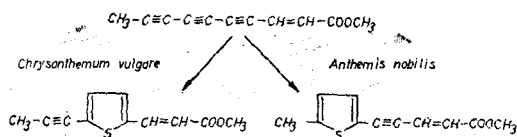
The compounds isolated from the avocado-pear, which have an acetylene bond at the end of the molecule can also be classified among these compounds.

The alkynylthiophenes also belong to the heterocyclic substituted polyacetylenes. They have been found in many plants and usually have the unsaturated chain at the two-position, they often have a propynyl or a butynyl group at the five-position.



prompted us to investigate the addition of H₂S to the diyne group.

Compounds with a diyne group react readily with H₂S in weakly alkaline media to give a good yield of 2,5-substituted thiophenes. In vitro, the reaction proceeds if cysteine or glutathione are used instead of H₂S. A similar reaction may lead to the formation of thiophenes in the plant cell, since we have found 2-¹⁴C-propynylthienylacrylic acid ester after feeding 2-¹⁴C-dehydromatricaria ester to *Chrysanthemum vulgare*.



Offering ³⁵S-cysteine lead to incorporation of the sulfur into alkynylthiophenes. In contrast to *Chrysanthemum vulgare*, *Anthemis nobilis* is able to form 5-methylthienylpentynenic acid ester from dehydromatricaria ester. This biogenetic relationship of the alkynylthiophenes to the polyacetylenes with the same or different number of carbon atom has been demonstrated in many other examples.

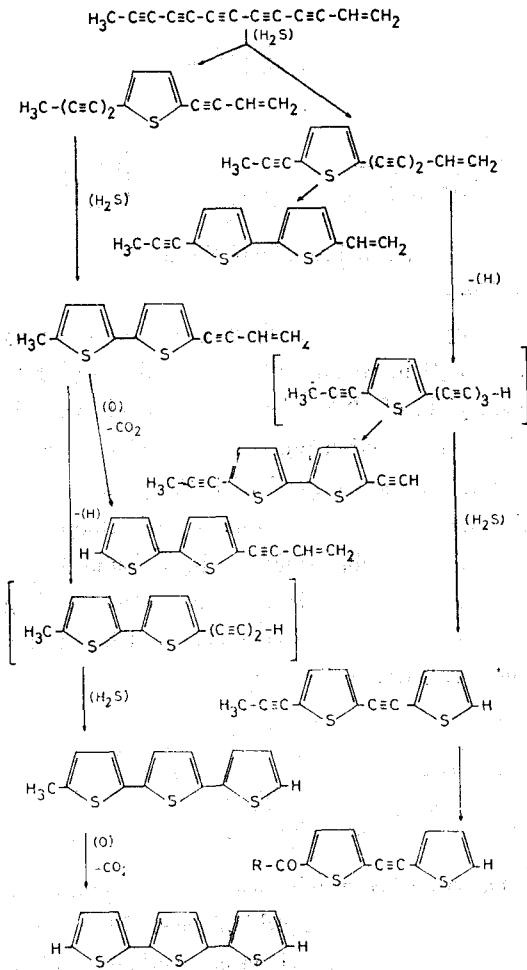
For instance, Bohlmann and others demonstrated in feeding experiments that alpha-terthienyl is formed from tridecenpentaene via a number of steps.

The spiroketalenolesters discovered by Bohlmann in 1965 contain a new type of bicyclic ring system. Since then many of these enoether-poly-yne have been found. Feeding experiments have clarified the biosynthesis of these com-

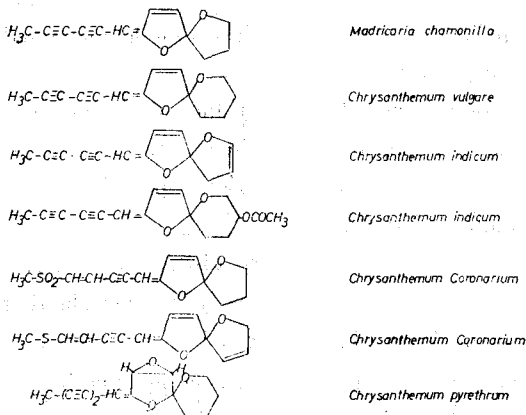
The red, unstable, dithioketoalkynes could be the precursors of alkynylthiophenes. α-Terthienyl, which has been described by Zechmeister, and bithienyl derivatives occur in species of *Tagetes*. These thiophenes, which occur in various African species of *Tagetes*, are active against nematodes.

Interesting substances are found in this class; substances such as propynylthienylacrylic acid ester and junipal, for which we described the first syntheses.

Alkynylthiophenes occur together with polyacetylenes in plants. Some of them have the same number of carbon atoms, have a diyne or triyne chromophore, and differ only in the formal addition of one molecule of H₂S. This observation



pounds. They are formed from oleic acid via a number of intermediate steps, which may be related to the previously mentioned reaction



Antibiotically Effective Acetylene Compounds Found in Nature

Name	Structure	Occurrence
Aquarymycin	$\text{H}_2\text{NOC}-\text{C}\equiv\text{C}-\text{CONH}_2$	<i>Streptomyces reticula</i> var. <i>aquarymyceticus</i> , Actinomycetales
Mycomycin	$\text{HC}\equiv\text{C}-\text{C}\equiv\text{C}-\text{CH}=\text{CH}-\text{CH}=\text{CH}-\text{CH}=\text{CH}-\text{COOH}$	<i>Noradonia acidophilus</i> , Actinomycetales
Agrocybin	$\text{CH}_2(\text{C}\equiv\text{C})_2-\text{CONH}_2$	<i>Agrocybe dura</i> , Agaricaceae
Diatretylene II	$\text{HOOC}-\text{CH}=\text{CH}-(\text{C}\equiv\text{C})_2-\text{CN}$	<i>Ciliocybe diatrete</i> , Basidiomycetales
Diatretylene amide	$\text{HOOC}-\text{CH}=\text{CH}-(\text{C}\equiv\text{C})_2-\text{CONH}_2$	<i>Ciliocybe diatrete</i> , Basidiomycetales
Nematrin	$\text{HC}\equiv\text{C}-\text{C}\equiv\text{C}-\text{CH}=\text{C}=\text{CH}-\text{CH}(\text{O})-\text{CH}_2-\text{CH}_2$	<i>Paria tenuis</i> , <i>P. corticula</i> et al. Basidiomycetales
Nematonic acid	$\text{HC}\equiv\text{C}-\text{C}\equiv\text{C}-\text{CH}=\text{C}=\text{CH}-\text{CHOH}-\text{CH}_2-\text{COOH}$	<i>Paria tenuis</i> , <i>P. corticula</i> et al. Basidiomycetales
Biformin	$\text{HC}\equiv\text{C}-(\text{C}\equiv\text{C})_2-\text{CH}_2-\text{CH}-\text{CH}_2\text{OH}$	<i>Polyporus biformis</i> , Basidiomycetales
Diatretylene III	$\text{HOCH}_2\text{C}\equiv\text{C}\text{C}\equiv\text{C}\text{CH}=\text{CH}-\text{COOH}$	<i>Pleurotus ulmarius</i> , <i>Tribolium panzerium</i> , <i>Ciliocybe diatrete</i> , Basidiomycetales
Marasin	$\text{HC}\equiv\text{C}-\text{C}\equiv\text{C}-\text{CH}=\text{C}=\text{CH}-\text{CH}_2-\text{CH}_2\text{OH}$	<i>Marasmius remealis</i>
Capillin	$\text{C}_6\text{H}_5-\text{CC}(\text{C}\equiv\text{C})_2-\text{CH}_3$	<i>Chrysanthemum fulvescens</i> L., <i>Artemisia capillaris</i> L., Compositae, <i>Agropyrum repens</i> L., Gramineae
Calinacetyde	$\text{C}_6\text{H}_5-\text{CH}_2-\text{C}\equiv\text{C}-\text{C}_6\text{H}_5$	<i>Carlina acaulis</i> L., Compositae

steps

I have collected some of the more interesting polyacetylenes which have antibiotic properties in the next slide. Acetylenedicarboxamide is said to have cytostatic actions. Mycomycin is said to have tuberculostatic properties, it has an allene group conjugated with an acetylene group and is very unstable. Mycomycin is too toxic to be used therapeutically.

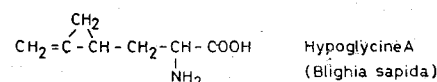
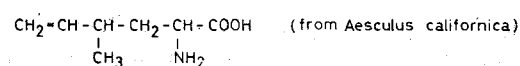
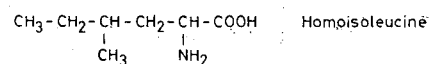
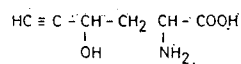
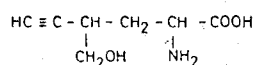
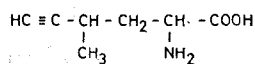
As well as the polyacetylenes which have been mentioned, which mainly have an unbranched chain, acetylene compounds with branched chains have been found in recent years.

Whereas the biosynthesis of the straight chain polyacetylenes probably starts from oleic acid, that of the branched-chain compounds suggests that they belong to a different class of naturally-occurring substances.

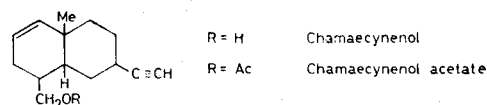
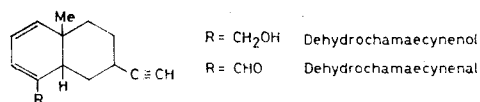
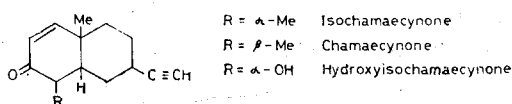
I had already mentioned freelingin among the alkynylfurans-it has a methyl branching.

In 1969 there amino acids were isolated from the seeds of *Euphorbia longan*; some of the amino acids have a branched chain and a terminal acetylene bond. These amino acids could have a close biogenetic relationship to homoisoleucine or 2-amino-4-methylhexenoic acid, whi-

Acetylenic Amino Acids from *Euphoria Longan*

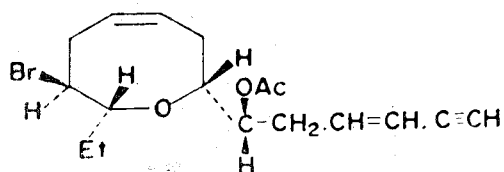
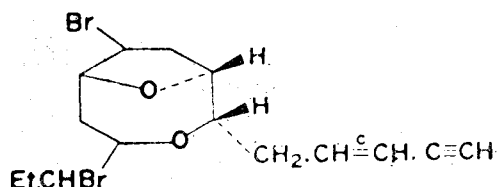
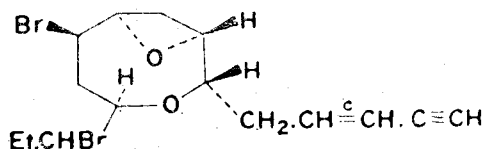


ch have been found in seeds of the closely related *Aesculus californica*, or to hypoglycin A, a beta (methylenecyclopropyl) alanin, which occurs in the fruits of a bush in the hippocastanaceae family which grows in West Africa and South Florida.



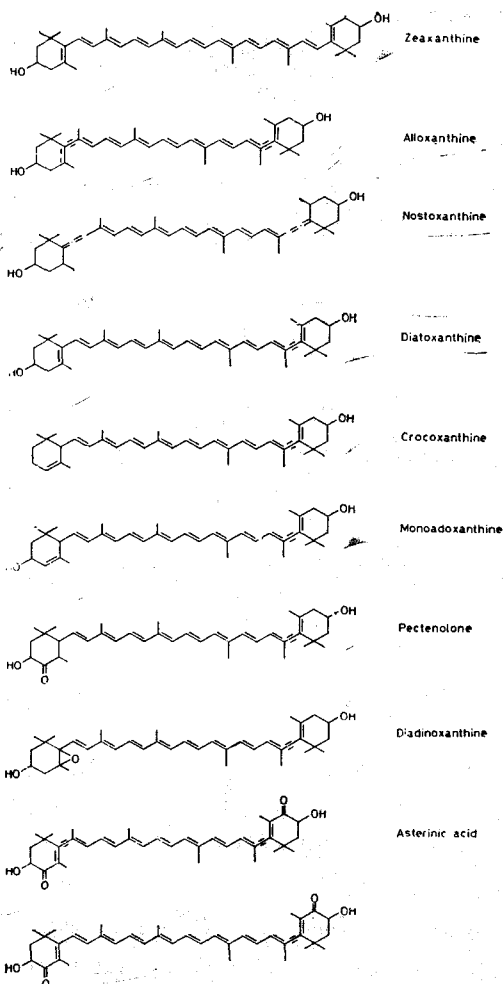
Some of the substances in cypresses can be classed as bicyclic sesquiterpenes, they also have an ethynyl side-chain. They could have a bio-

genetic relationship to eudesman and could be regarded as noreudesmans.



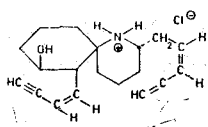
The bromine-containing, 8-membered cyclic ethers with a pentynyl side chain, which have been isolated from red algae have a novel structure. These compounds are laureatin, Isolaureatin and laurencin. An unsaturated fifteen-carbon alcohol could be the biogenetic precursor from which the cyclic ethers are formed, via an epoxide.

In 1967 the first carotinoid with two acetylene bonds was found. It was alloxanthin, a 7,8,7,8-tetrahydrozeaxanthin, which was isolated from algae, along with some other xanthophylls. Since then other 7-acetylene carotinoids have been identified in the pigments of algae and also in marine animals, such as the giant clam and in starfish. The acetylene carotinoids usually occur in small quantities and together with carotinoids with allene bonds and the corresponding xanthophylls with a polyene chain. There must be a biogenetic relationship to the xanthophylls, but neither this question

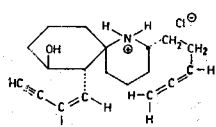


nor their place in the intermediary metabolism of the cell has been answered.

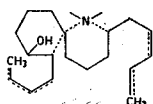
The first substances with an acetylene bond



Histronicotoxin · HCl



Dihydro-iso-histronicotoxin · HCl



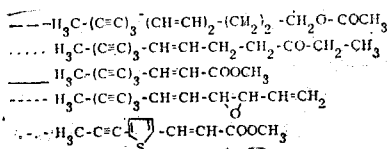
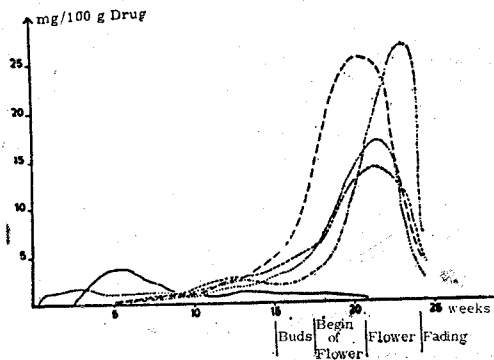
Hypothetical C₁₉ Precursor

that were isolated from animals are histrionicotoxin and dihydro-iso-histronicotoxin. These are azaspiro undecanes with a butenynyl and pentenynyl side-chain—they are the toxic principles of the frog *Dendrobates histrionicus*. It is most probable that this substance does not have an exogenous origin. A straight-chain nineteen-carbon precursor has been proposed for its biosynthesis.

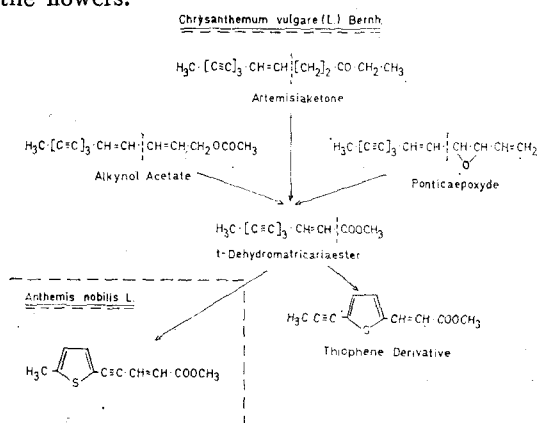
Although biosynthetic pathways have been proposed for most of the known polyacetylenes on the basis of feeding experiments, certain questions remain concerning this interesting group of compounds. When are these substances formed in the course of the vegetative period of the plant, and what is their role?

What is the reaction by which the plant cell introduces the triple bond? What intermediate steps are required for this reaction? What is the relationship of these reactive substances to the intermediary metabolism of the cell, are they precursors of primary and secondary plant constituents?

I will briefly discuss some of the experimental results and imaginative hypotheses related to these questions.



Two days after showing polyacetylene-free seeds of *Chrysanthemum vulgare*, whose roots contain five polyacetylenes and one alkynyl thiophene, the seedlings contain artemisia ketone. After 2 1/2 weeks dehydromatricaria ester appears and after a further 2 1/2 weeks too polyacetylenes and alkynylthiophene can be identified. With the exception of dehydromatricariaester, the content of polyacetylenes increases during the vegetation period. The highest concentration is found at the time of flowering; after flowering, the concentration falls. The concentration of polyacetylenes remains constant during the rest period in winter, and then follows a pattern similar to that in the one year plant. Small quantities of a polyacetylene are sometimes seen in the shoots and small quantities of three of these compounds are found in the flowers.



The order of appearance of the polyacetylenes, and the quantities in which they appear, make biogenetic relationships likely.

Feeding of *Chrysanthemum vulgare*, with unspecifically labelled substances has helped to clarify these relationships. I will not discuss all of the findings of these investigations; I will mention the main results. Of the six polyacetylenes in this plant, trans-dehydromatricaria ester seems to have a central position. It is

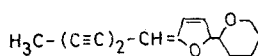
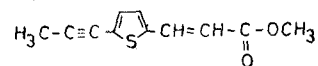
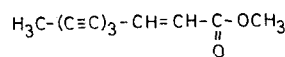
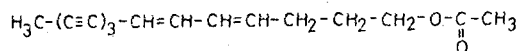
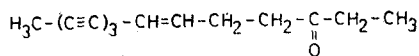
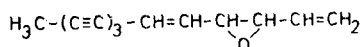
formed after the feeding of ^{14}C -artemisia ketone, ^{14}C -alkynol acetate, and ^{13}C -ponticaepoxide. The entryne group which is common to the four polyacetylenes remains intact during these reactions (see formula scheme).

On the other hand, trans-dehydromatricaria ester leads to the formation of trans-5-propynyl-thienylacrylic acid -2-methyl-ester, as was mentioned previously. After feeding (2- ^{14}C) -trans-dehydromatricaria-ester, only alkynylthiophene was radio-active. No incorporation resulted from the feeding of ^{13}C -pontica epoxide, ^{14}C -artemisia ketone or ^{14}C -alkynol acetate.

Feeding experiments which are performed with whole plants have a number of disadvantages for example, hydrocultures cannot be kept sterile. Therefore, one cannot exclude that micro-organisms may have metabolized some of the offered ^{14}C -acetate, before it has been absorbed by the plant. Feeding experiments in whole plants cannot provide evidence regarding the catabolism of secondary plant substances such as the thiophen-derivatives or the polyacetylenes.

This is why we have attempted to use sterile

Polyacetylenes of *Chrysanthemum vulgare* (L.)



Feeding of ^{14}C of ^{35}S Precursors to Cell Cultures of *Chrysanthemum vulgare* (L.) Bernh.

Fed substance	Ponticae- poxyd	Alkino- lacetate	Artemi- siaketon	tr-Dehy- droma- tricar- iaester	Alkynyl- thiophen	Enolethe rpolyin	new com- pounds	unsaturated fatty acids		
								Oleic acid	Linolic acid	Linoleic acid
$1\text{-}^{14}\text{C}$ -Acetate	+	+	+	+	-	-	-	+	+	+
^{35}S -Cysteine	-	-	(+)	-	-	-	(+) ^a			
^{35}S -MgSO ₄ +tr-Dehy- dromatricariaester	(#)	(+)	(#)	(#)	-	-	+ ^b + ^c			
Ponticaepoxyd	#	#	#	(+)	-	-	-			
	8.43%	6.1%	2.83%							
Alkinolacetate	(#)	#	#	(+)	-	-	-			
		7.88%	4.79%							
Artemisiaketon	#	#	#	(+)	-	-	-			
	20.77%	10.71%	18.81%							
$\text{U-}^{14}\text{C}$ -Oleic acid	+	+	+	+	-	-	-	+	+	+
	1.92%	0.96%	0.50%							

() : no radio-active

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New Compounds: a: Trideca-triin (7,9,11)-trien (1,3,5) b: 325-compound c: 335-compound

cell cultures for these experiments. Cell cultures of *Chrysanthemum vulgare* form small amounts of the four polyacetylenes which are found in the whole plant. The formation of these polyacetylenes is dependent on the nutrients in the medium. It has not been possible to prepare cell cultures in which the thiophene derivative or enolether polyyn could be identified.

Addition of [$1\text{-}^{14}\text{C}$] acetate to the medium of the cell culture leads, after about 10 days growth to radioactively labelled polyacetylenes and eighteen-carbon unsaturated fatty acids. The same polyacetylenes are formed when labelled oleic acid is added to the medium. We have been unable to prepare large quantities of labelled trans-dehydromatricaria ester by this method, nor to incorporate radioactivity into thiophene derivatives or enoletherpolyynes, even with variation of the experimental conditions.

When cysteine was offered, only arthemisia ketone and a new compound could be isolated. The new compound is trideca-triyn-triene, which could be the precursor of pontica epoxide.

After feeding of t-dehydromatricaria ester and Mg³⁵SO₄, the four polyacetylenes can be isolated unlabelled together with small quantities of two

labelled compounds whose structures we have not been able to determine.

Radioactively labelled alkynolacetate and arthemisia ketone are formed in cell cultures from labelled pontica epoxide. Trans-dehydromatricaria ester is not labelled.

Radioactively labelled alkynol acetate yields radioactive arthemisia ketone; but pontica epoxide and trans-dehydromatricaria ester are not labelled.

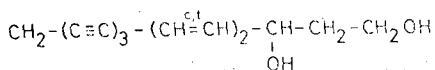
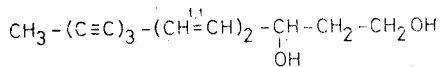
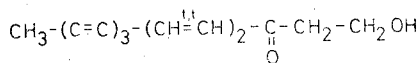
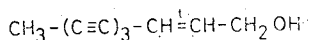
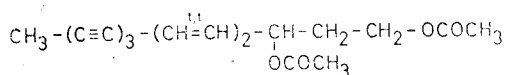
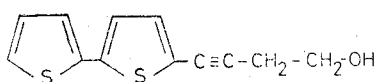
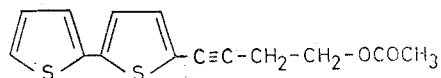
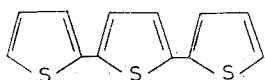
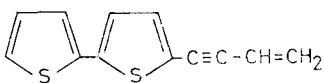
Arthemisia ketone yields radioactive pontica epoxide and alkynol acetate in the cell culture, where as t-dehydromatricaria ester is once again inactive.

These three feeding experiments with cell cultures suggest the same biogenetic relationships as those suggested by feeding experiments in the whole plant.

The cell culture cannot metabolize propynthienyl arylacid ester, as experiments have shown in which was added to the sterile medium.

We have also prepared cell cultures of *Tagetes erecta*. These cell cultures for four thiophene derivatives, which are also found in underground parts of this plant. But none of the poly-

Tagetes erecta



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cetylenes which are found in the plant could be found in the cell culture. Feeding with [1-¹⁴C] acetate did not lead to the incorporation of ra-

Feeding of ¹⁴C or ³⁵S Precursors to Cell Cultures of *Tagetes erecta*.

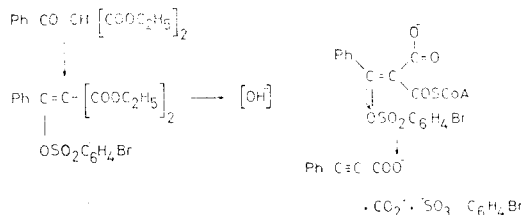
Fed substance	Thiophenes				Polyacetylenes	Unsaturated fatty acids		
	Bithienylbutine	α-Terthienyl	Bithienylbutinolate	Bithienylbutinol		Oleic acid	Linolic acid	Linoleic acid
1- ¹⁴ C-Acetate	—	—	‡ 3.12%	—	—	+	‡	+
Mg ³⁵ SO ₄	‡ 0.424%	+	‡ 0.731%	+	—	—	—	—
³⁵ S-Cysteine HCl	‡ 0.279%	±	+	±	—	—	—	—
U ¹⁴ C-Oleic acid	‡ 0.019%	+	‡ 0.021%	+	—	‡	‡	+

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dioactivity into thiophenes or polyacetylenes. But ³⁵S offered in the form of MgSO₄ or cystein is incorporated into the thiophene ring.

The addition of labelled oleic acid to the cell cultures gives rise to radioactively labelled thiophene derivatives, and the lipid phase of the cells contains radioactively labelled linolic acid and linoleic acid.

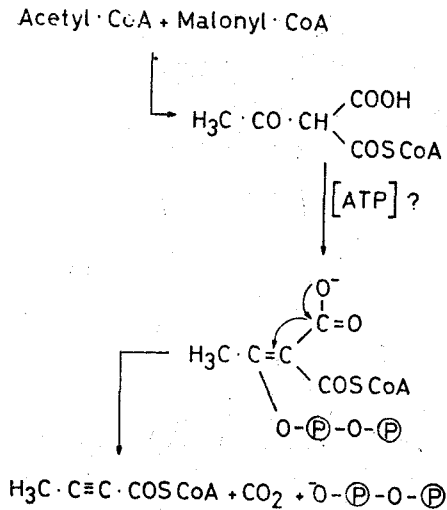
α-Terthienyl is not metabolized by the cell cultures of *Tagetes erecta*, but bithienylbutanol is formed from bithienylbutynene.



Central to the question of how polyacetylenes are formed in the problem of the formation of the triple bond. Jones regards acetylmalonate-CoA as a precursor for acetylene bonds. He suppose that first an enol-ester derivative of acetylmalonic acid is formed, perhaps with the help of ATP, and that this derivative simultaneously undergoes decarboxylation and elimination of a pyrophosphate group to form the triple bond.

A direct dehydration of acetylacetate to a butynyl group is less likely, as this requires a

lot of energy. This scheme is supposed by the observation of Fleming and Harley-Mason that the enolsulfonate of benzoylmalonic acid ester is converted into phenylpropynic acid by treatment with 0.2 molar sodium hydroxide in dioxane at room temperature.



The feeding experiments with oleic acid supported the assumption that the triple bond can be introduced in the longer aliphatic chains of a fatty acids. An enzyme system that catalyses dehydrogenation of an ethylene bond is as yet not known.

Similarly, nothing is known about the position of polyacetylenes in the intermediary metabolism, and their possible relationship to the primary and secondary constituents of the plant. Ladies and gentlemen, at this point I should like to end my talk; the picture of this class of naturally-occurring substances that I have given you is necessarily incomplete. But perhaps I have been able to give the impression that this class of compounds deserves attention from various aspects.

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