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Antineoplastic Natural Products and the Analogues VIII Synthesis of some Coumarins and Their cytotoxic Activities on L1210 Cell

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Abstract□Some coumarins were sythesized for the screening of their cytotoxic activities against L1210 cell. Of the coumarins sythesized, 6,7-dihydroxycoumarin (esculetin) and 7,8-dihydroxycoumain (daphnetin) as coumarins with dioxygenated A-ring, and 6-acetoxy-5,7-dimethoxycoumarin and 5,7-dimethoxy-6-hydroxycoumarin as trioxygenated ones, show considerable cytotoxic activities, ED 50 being 4.3, 8.8, 17.2 and 5.5 μg/ml in the same order as the substances.

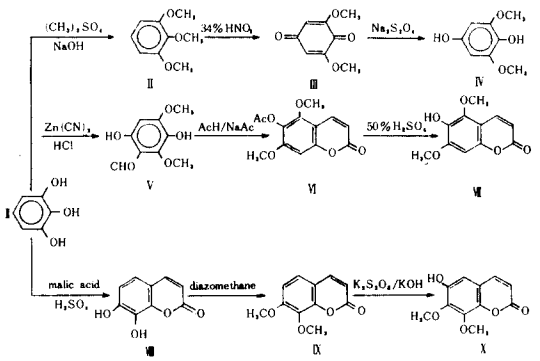
The extent of oxygenation of the A-ring and the positions of the oxygen functions eventually play an important role for the cytotoxic activity.

Keywords□Coumarin structure, Cytotoxicity, L1210 cell.

We have previously reported that aurapten, 7-geranyloxycoumarin, is cytotoxic on the L1210 cell *in vitro*[1]

Geraniol and 7-hydroxycoumarin were obtained by its hydrolysis. It was found that geraniol only shows the cytotoxic activity, while 7-hydroxycoumarin did not. It was therefor assumed that the coumarin part functions as a carrier of geraniol. The usefullness of coumarins as carrier group of antitumor agents has been studied.[2,3,4]

For the purpose of supplying carrier moieties of geraniol we have synthesized various coumarins. In the course of screening of the synthesized coumarins for the cytotoxic activity we have found that some ot those are active in fact.



Scheme 1. Synthesis of Coumarins

EXPERIMENTAL METHODS

Melting points were measured by the capillary methods and not corrected. IR-spectra were taken on Perkin-Elmer Moedl 783 Infrared Spectrophotometer. NMR spectra were rocorded on Varian 60A and FT-80 machine, using TMS as internal standard.

Biological Methods

The culture and maintenance of the L1210 cell, and the pocedures for determination of ED50 values were described elsewhere.[1]

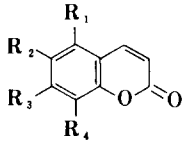
Synthesis of coumarins (scheme 1)

2,6-dimethoxyhydroquinone (IV)—This was sythesized by Baker's method.[5]

3.6-dihydroxy-2,4-dimethoxybenzaldehyde (V) -3.4g of IV were dissolved in dried ether and 7g of fine powdered zinc cyanide added to the solution. Then dried HCl gas was streamed into the mixture with stirring for 90 min, followed by evaporation of ether. The residue was dissolved in 150ml of water and heated at 100°C for an hour. After cooling this mixture, the sedimented solid was filtered and washed with cold water. The solid filter cake was recrystallised in ether/petroleum ether mixture. Yield: 1.5g of V.

5,7-dimethoxy-6-acetoxycoumarin (VI) and *5,7-dimethoxy-6-hydroxycoumarin (VII)*-1.5g of V was dissolved in 12.5 ml of acetic ahydride. 1.5g of sod. acetate were added into the solution and heated at 160-170C for 24 hrs. After cooling, the reaction mixture was shaken out with chloroform. The cholroform extract was chromatographed over a silica gel column ($CHCl_3$, 3 x 15 cm). The corresponding fraction (identified with IR)

Table I. NMR data of the synthesized coumarins



Compounds	Substitution				H-NMR (ppm)								
	R_1	R_2	R_3	R_4	Solvent	3-H	4-H	5-H	6-H	8-H	OMe	OAc	OH
6-acethoxy-5,7-dimethoxycoumarin	OCH_3	OAc	OCH_3	H	$CDCl_3$	d6.30	d7.95			s6.70	s3.95	s2.38	
6-hydroxy-5,7-dimethoxycoumarin	OCH_3	OH	OCH_3	H	$CDCl_3$	d6.28	d8.00			s6.65	s4.00		s5.65
7,8-dimethoxy coumarin	H	H	H	OCH_3	OCH_3	$CDCl_3$	d6.27	d.7.70	d6.92	d7.28		s4.00		
6-hydroxy 7,8-dimethoxycoumarin	H		OH	OCH_3	OCH_3	$CDCl_3$	d6.39	d7.69	s6.81			s4.12		s5.95

s = singlet, d = doublet

was recrystallised in ether. Yield: 300mg of VI.

VI (300mg) was deacetylated in 50% sulfuric acid at 70°C. The deacetylated product was recrystallised from methanol. Yield: 210 mg of VII.

7,8-dihydroxycoumarin (VIII) and 7,8-dimethoxycoumarin (IX)-VIII was synthesized by means of Pechmann's method.[6] VII was methylated with diazomethane to obtain IX.

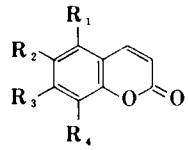
6-hydroxy-7,8-dimethoxycoumarin (X)-1g of IX and 0.1g of $FeSO_4$ were dissolved in 20ml of 5% KOH solution. 90ml of 3.2% $K_2S_2O_8$ solution was slowly added under stirring. After the addition, the reaction mixture was stirred for an hour. After acidifying the reaction mixture with diluted sulfuric acid and extracting the unreacted starting material with ether, the water phase was refluxed for an hour. After cooling it was extracted with ether. After evaporating ether, the residue was recrystallized from methanol. 200mg of X.

The NMR data of the coumarins were shown in Table I.

RESULTS AND DISCUSSION

As shown in scheme 1, IV was transformed to the

Table II. Coumarins and their ED50 values on L1210 cell



Coumarins	R_1	R_2	R_3	R_4	ED50 $\mu g/ml$
Umbelliferone	H	H	OH	H	>20.0
7-methoxycoumarin	H	H	OCH_3	H	>20.0
7-allyloxycoumarin	H	H	Oallyl	H	>20.0
7,8-dihydroxcoumarin (VIII)	H	H	OH	OH	8.8
7,8-dimethoxycoumarin (IX)	H	H	OCH_3	OCH_3	>20.0
7,8-dimethoxy-6-hydroxycoumarin	H	OH	OCH_3	OCH_3	>20.0
6,7-dihydroxycoumarin	H	OH	OH	H	4.3
6-acetoxy-5,7-dimethoxycoumarin	OCH_3	OAc	OCH_3	H	17.2
5,7-dimethoxy-6-hydroxycoumarin	OCH_3	OH	OCH_3	H	5.5
Aurapten (7-geranyloxycoumarin)	H	H	O-geranyl	H	10.2
Geraniol					6.5

aldehyde (V) by the Gatterman reaction which was condensed to VI by the Perkin method. VIII, known as daphnetin, was obtained by means of Pechmann's method. Under conditions of Elbs reaction this was methylated and hydroxylated to obtain X. The yields were enough to carry out our present studies.

The structure and ED50 values of the coumarins synthesized were designated in Table II. As already reported,[1] 7-hydroxycoumarin (umbelliferone) and its alkylated derivatives had no activities.

Among coumarins with dioxygenated A-ring, both of 6.7-dihydroxy-and 7,8-dihydroxycoumarins showed good cytotoxicity. But methylated products were inactive.

Also coumarins with trioxygenated A-ring such as 6-acetoxy-5,7-dimethoxycoumarin and 5,7-dimethoxy-6-hydroxycoumarin showed activity, the former being less active. However, 6,7,8-trioxygenated coumarin such as 7,8-dimethoxy-6-hydroxycoumarin did not.

It is apparent from Table 2 that the cytotoxic activity is dependent upon the number of hydroxy groups, their position and the extent of alkylation or acylation.

Besides the drug carrying functions, some coumarins exert antimetastatic[7] as well as antitumor activities.[8-12]

From these results we could design to synthesize the coumarin derivatives that function as cytotoxic, antimetastatic as well as drug carrier.

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