생 약 학 회 지
Kor. J. Pharmacog.
11(1):15~23(1980)

Iridoid Glycoside (I)

Studies on the Iridoid Glycoside of *Ajuga spectabilis* Nakai

Bo-Sup Chung, Hyung-Kyu Lee, and Jin-Woong Kim
College of Pharmacy, Seoul National University

이리도이드 配糖體 (1)

자란초의 이리도이드 배당체

정 보 섭·이 형 규·김 진 웅
서울대학교 약학대학

A new iridoid glucoside was isolated from the whole plant of *Ajuga spectabilis* Nakai (Jaran-cho; Labiatae). This compound was obtained as white plate-like crystal and named as Jaranidoside. It has a molecular formula $C_{17}H_{26}O_{12}$ and mp 128~130°C. The structure of the Jaranidoside was assumed from data of chemical reactions and PMR specturum of the compound. To determine the most favorable conformation, informations on the proton coupling and chemical shift were used.

Jaranidoside exhibited a stimulating activity on smooth muscle and cardiac muscle. No antimicrobial activity on five microorganism strains was observed.

Introduction

The structure and physiological activities of iridoid compounds from plants have been the subject of numerous investigations. Plant iridoid compounds can be classified as follows[1].

1. Methylcyclopentanoid monoterpenes of the nepetalactone type
2. Iridoids
   a) iridoid glucosides
   b) non-glucosidic iridoids
3. Monoterpene alkaloids
4. Secoirdoids
5. Non-tryptophan portions of different indole and isoquinoline alkaloids

Most of the iridoid compounds exist as glycosides and degenrative terpenes are thought as agycone moieties of the glycosides[2].

In general, iridoid glycoside has a very stong bitter taste. The aglycone moiety of the glycoside is very unstable, and upon hydrolyzation with an enzyme or an acidic solution, the color of the glycoside rapidly changes from colorless to bluish violet. If heat is applied further, black resinous precipitates can be obtained. It has also been noted that the dried plant such as *Rhemannia glutinosa* Lib. var. *purpurea* Mak. or *Scrophularia buergeriana* Miq. which has iridoid glycoside as a chemical component gradually turns to black during storage[2].

Secoiridoid glycosides which are formed by the rupture of the cyclopentane ring of the iridoids are one of the components of crude drug used as a stomachics. It was reported that secoiridoid glycosides showed a stimulating activity on the excretion of gastric juices and biles without any toxicity[2]. Also it was known that

some iridoid compousnds among monoterpene lactones excited cats and other Felidae animals whereas some strongly attracted insects such as lacewings[3,4].

Iridoids exhibited a very diverse physiological activity[1], that is, antimicrobial activity (aucubin, aguside, asperuloside, plumericin, isoplumericin, oleuropein, fulvoplumierin), hypotensive effect (oleuropein), analetic and antiphlogistic property (harpagoside), sedative agent (valepotriates—valtratum, acevaltratum, didrovaltratum), laxative property (geniposide), purgative activity (geniposide, plumieride, verbenalin, loganin, gardenoside), antileukemic activity (allamandine), various other effects (catalposide, catalpol: diuretic principle, aucubin: the uric acid excretion of the kidney).

However, Ajuga genus in Labiatae plants studied in limitation, and the presence of iridoids such as ajugoside, harpagide, acetyl parpagide, ajugol (leonuride), and reptoside were only reported[5].

In view of the important diverse physiological activities of iridoids, it is thought that chemical and pharmacological characterizations of a Korean native plant in Ajuga genus of Labiatae family will shed a light to develop a new drug from natural sources.

From this purpose *Ajuga spectabilis* Nakai (Jaran-cho) which was wildly found at Baek-Bong was chosen for the present study. The Jaran-cho is a perennial herb and has wide-ellipsoidal leaves and deep violet flowers[6,7].

The present study directed toward the clarification of the chemical components of the Jaran-cho and the estimation of its pharmacological activities.

Experimental & Results

1) Material and Apparatus

a) Material

Ajuga spectabilis Nakai was collected in May and June at Baek-bong (Pyeongnae, Kyeongki-Do) and dried for the experiment.

b) Apparatus

Melting point apparatus: Gallenkamp (uncorrected)

Elemental analysis: Coleman Model 33 PMR spectrum: Varian EM-360 (60 MHz), Perkin-Elmer R-32 (90 MHz)

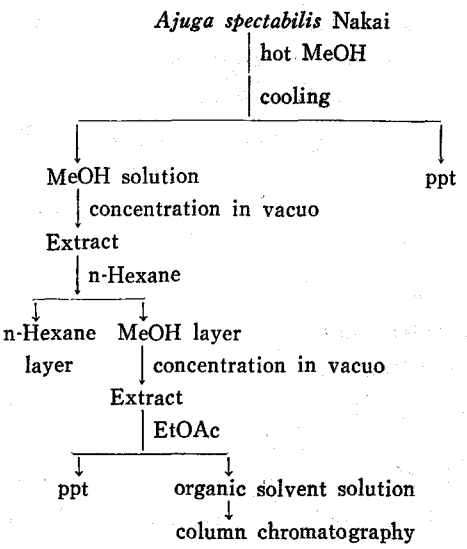
IR spectrum: Beckman IR-20A

2) Extraction and Isolation

The main procedure of the extraction of iridoid compounds from Jaran-cho was shown in Scheme 1.

800 grams of the dried plant was extracted with 2*l* methanol (technical grade) five times and the extract was left overnight at room temperature to precipitate the resinous compounds. After removal of the precipitate, the MeOH extract was concentrated under vacuum to one twelvth of its volume. In order to remove non-polar compounds, the MeOH extract was treated with 300ml n-Hexane five times and Hexane layer was discarded.

The resulting MeOH layer was concentrated until half of its volume under reduced pressure.



Scheme 1. Extraction and separation

Equal volume of EtOAc was added to precitate very polar constituents out and the resulting soluton was concentrated to dryness under vacuum. The resulting residue was then subjected to chromatography for the fractionation of the components.

It was noted that both of the extraction with cold and hot MeOH gave two major spots on TLC without showing any difference. According to the TLC result, isolation of these two compounds in the extract was attempted by column chromatography.

The residue was chromatographed using column(4×40cm, 5×50cm) and silica gel G(230 mesh) as an adsorbent and $CHCl_3$-benzene-MeOH(4-4-1) as an eluting solvent system. The fraction which corresponded to a iridoid compound according to the TLC result was collected and purified by rechromatography using column (3×8cm) and silica gel G(230 mesh) as an adsorbent and cyclohexane benzene-$CHCl_3$-MeOH (10-10-25-5) as an eluting solvent system.

It was shown that the rechromatographed fraction gave only one single spot indicating the fraction was very pure. When the fraction was recrystalized with $CHCl_3$+cyclohexane mixture, white plate-like crystals were obtained.

3) Analysis

a) Chemical reactions

The compound was characterized by anthron test, Liebermann-Buchard test and $SbCl_3$ reactions, and the results are as follows:

Anthron test: positive(blue-green)

Liebermann-Buchard test: positive(red)

$SbCl_3$ reagent($CHCl_3$ solution): from pink to blue-green (added into MeOH solution)

$SbCl_3$ spray reagent: from pink to blue (on TLC)

b) Acetylation

The compound was subjected to acetylation in order to determine the number and position of primary and secondary hyndroxyl groups by instrumental analysis.

The compound(100mg) was dissolved in 3ml pyridine and 4ml acetic anhydride was added to the pyridine solution, and the miture was left overnight at room temperature. The mixture was poured into 200ml distilled water and then extracted with 100ml $CHCl_3$ three times. The $CHCl_3$ layer was collected and washed with distilled water and dried over anhydrous Na_2SO_4. Then the $CHCl_3$ solution was evaporated until dryness under reduced pressure. This compound showed single spot on TLC (Rf value: 0.7, solvent system: cyclohexane-benzene $CHCl_3$-MeOH; 10-15-25-5).

c) Instrumental analyses

The compound was subjected to elementary analysis in order to get the empirical molecular formula. Results of the analysis are as follows.

Calculated; C 47.33% H 6.26%

Found; C 47.56% H 6.48%

empirical molecular formula; $C_{17}H_{26}O_{12}$

PMR data of the compound showed the typical chemical shift pattern of the iridoid compound as shown in Fig. 1.

6.40 ppm(d. J=6Hz, 1H, C_3-H)

5.95 ppm(s. 1H, C_1-H)

2.65 ppm(s. 1H, C_9-H)

2.05 ppm(s. 3H, CH_3CO-)

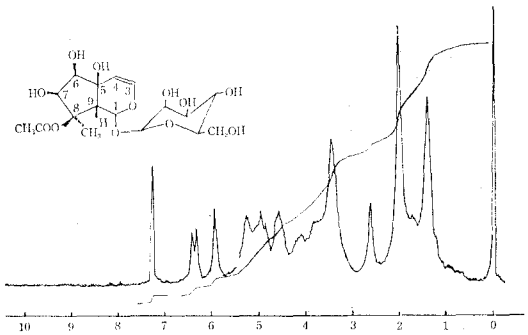


Fig. 1. PMR spectrum of the compound in $CDCl_3$ (60 MHz, internal TMS standard)

1.42 ppm(s. 3H, C_8-CH_3)

IR(film on KBr disc method) spectrum of the compound is shown in Fig. 2.

3400cm^{-1}(-OH)

1720cm^{-1}(C=O, ester)

1650, 750cm^{-1}(cis C=C)

1380cm^{-1}(-CH_3)

As shown in Fig. 3, PMR data with D_2O gave more detailed informations on chemical shift. (This chart is deshielded by 0.5ppm using external TMS standard.)

6.95 ppm(d. J=6 Hz, 1H, C_3-H)

6.42 ppm(s. 1H, C_1-H)

5.2 ppm(s. 1H, sugar C_1-H)

5.55 ppm(dd. J=6 Hz, 1.5Hz, C_4-H)

4.6-3.7 ppm(m. 8H, C_6-H, C_7-H and protons of sugar)

3.17 ppm(s. 1H, C_9-H)

2.55 ppm(s. 3H, CH_3CO-)

1.95 ppm(s. 3H, C_8-CH_3)

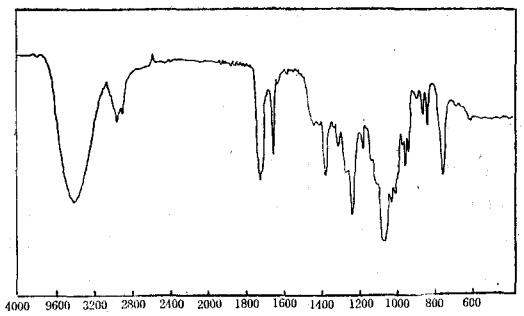


Fig. 2. Infrared spectrum of the compound (film on KBr disc)

PMR spectrum of the acetylated compound displayed seven acetate signals as shown in Fig. 4.

6.30 ppm(d. J-6Hz, 1H, C_3-H)

5.94 ppm(d. J-ca. 1.5Hz, 1H, C_1-H)

5.4-3.7 ppm(m. 10H, C_4-H, C_6-H, C_7-H, C'_1-H, C'_2-H, C'_3-H, C'_4-H, C'_5-H, C'_6-H)

2.75 ppm (d. J-ca. 1.5Hz, 1H, C_9-H)

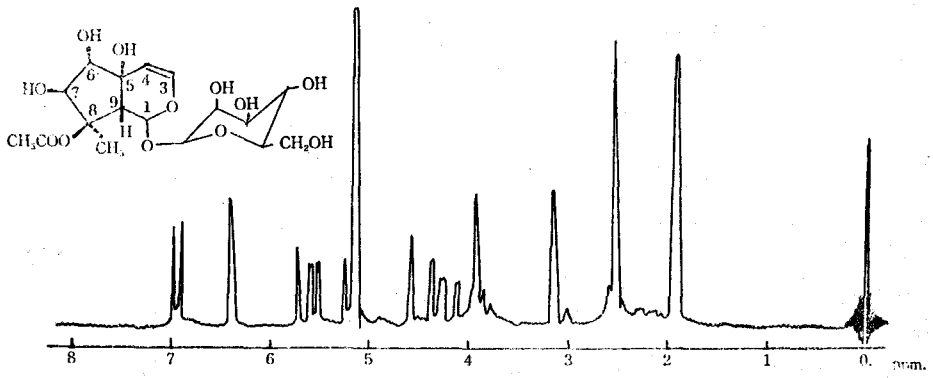


Fig. 3. PMR spectrum of the compound in D_2O (90 MHz, external TMS standard)

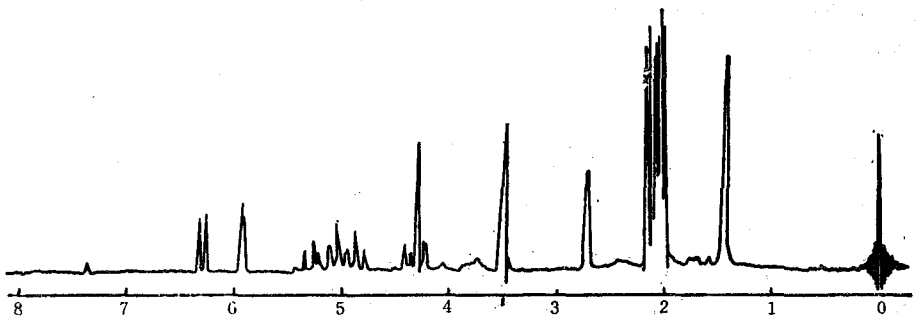


Fig. 4. PMR spectrum of the acetylated compound in $CDCl_3$(90 MHz, internal TMS standard)

2.2-2.0 ppm(21H, 7 CH_3CO-)

1.48 ppm(s. 3H, C_8-CH_3)

4) Hydrolyzation

The compound was hydrolyzed with 5% HCl solution as usual work and gave dark red resinous precipitate. The precipitate was not dissolved with any common organic solvent due to decomposition of the aglucone like other natural occuring iridoids. The reacted solution was neutrallized with $NaHCO_3$ and chromatographed with various solvent system. As compared with authentic samples using TLC, the sugar of the compound was identified with glucose.

5) Acetonization

The compound(20mg) in 1ml of dry acetone was added to 1.7ml acetone solution of $SnCl_2$ (500mg in 3ml). The solution was allowed to stand at room temperature under N_2 gas, stirring continuously, for about 2 hrs, and then it was treated with saturated $NaHCO_3$ aqueous solution. The mixture was filtered and the resulting solution was chromatographed using TLC. This gave two major spots of products(Rf value: 0.85, 0.75, original sample: 0.04 solvent system: cyclohexane-benzene-$CHCl_3$; 13-10-22-5)

6) Pharmacological activity

Results of the experiments for the contraction of the rat cardiac muscle by Langedorff apparatus and the contraction of the rabbit duodenum by Magnus apparatus are shown in Fig. 5 and Fig. 6. The each organ of animals was seperated and used just when animals died by cutting carotid artery.

Sample concentration: ca. 3 10^{-6}M in Kreb's Henseleit solution(pH 7.4 buffer, temp. 30°C)

the contraction of the rat cardiac muscle
: increased ca. 16.7%

the contraction of the rabbit duodenum
: increased ca. 20%

It was observed that if the sample concentration was raised 50 times of the above experimental concentration, the muscle contraction pattern exhibited irregularity and gradually diminished.

In order to screen the antimicrobial activity of the compound, five microorganism strains such as *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi* and *Pseudomonas aeruginosa* were employed. Results obtained with two sample concentrations, 2.5 microgram/ml, 10 microgram/ml, showed little antimicrobial activity of the compound on these five strains by the cup method. The inhibition circle of the compound was difficult of measuring diameters but to be. This experiment is thought to need more systematic study.

Medium: (20g agar+8g nutrition broth)/liter

Incubation: 37°C, for 20 hrs

Referance standard: Streptomycin sulfate(for injection made in Yu-Han Pharm. Co.)

Plate number: 4 per one microorganism strain

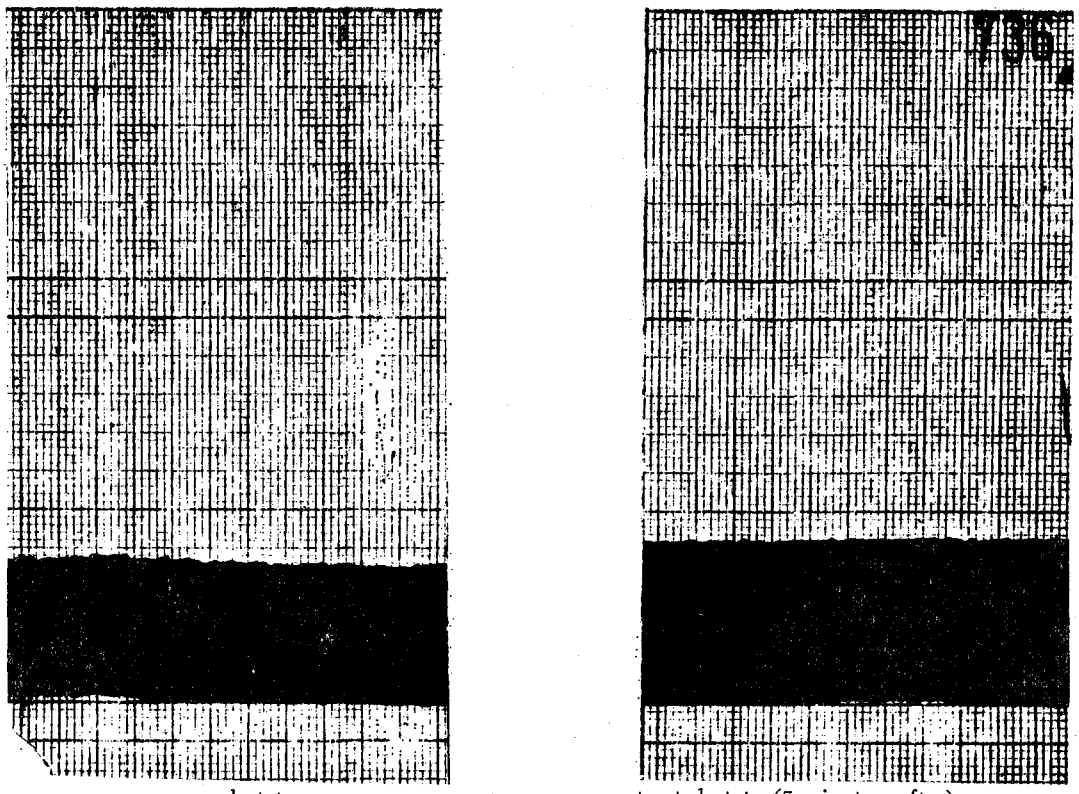
Measurement: diameter of growth inhibition circle

Discussion and Conclusion

Studies on the chemistry of the components of Jaran-cho revealed the presence of at least one new iridoid glycoside. This new compound is given a name as Jaranidoside in the present study.

The Jaranidoside was isolated from the substance which had a higher R_f value when the total extract of Jaran-cho was subjected to TLC. As mentioned in experimental part, the total extract showed two major spots. The substance which had a lower R_f value did not cover in this study because of the lack of the time.

The compound gave a positive reaction wtih Liebermann-Buchard test indicating the presence of terpene structure in its molecule. This assu-



normal state　　treated state (5 minutes, after)

Fig. 5. Kymographs of the effect of Jaranidoside on the cardiac muscle of the rat by Langendorff apparatus

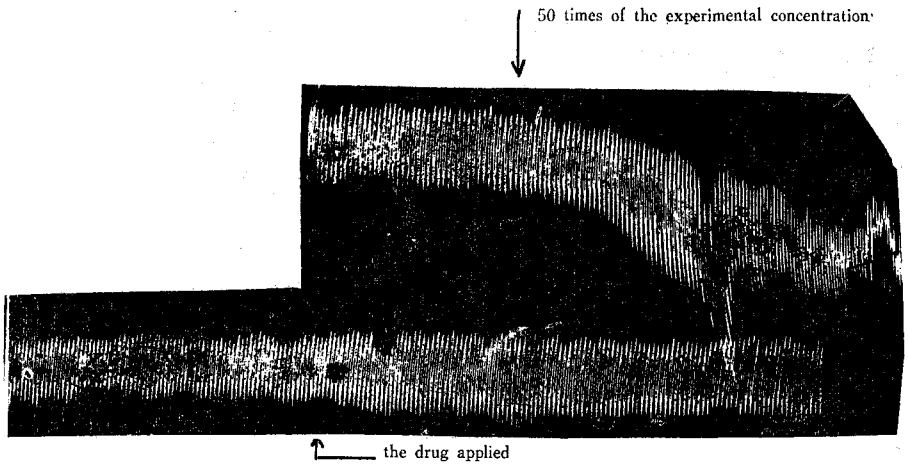


Fig. 6. Kymograph of the effect of Jaranidoside on the duodenum of the rabbit by Magnus apparatus

mption was further supported by $SbCl_3$ test which gave a blue color of the testing mixture assuring the presence of iridoid monoterpene. It also gave a positive reaction with anthron test supporting the molecule contained sugars. Thus, according to the results of the chemical-

Table 1. PMR data of iridoid glycosides of Jaranidoside analogs.

	C_1	C_3	C_4	C_5	C_6	C_7	C_8	C_9	CH_3
1	5.92	6.45	5.05	—	4.6	3.7	—	2.67	1.45
2	5.95	6.90	5.54	—	2.66	4.02	—	2.91	2.01
3	5.48~5.64	6.50	5.33	2.88~3.0	5.09	3.86~4.54	—	2.88~3.0	—
3*	4.80~5.27	6.33	4.80~5.27	2.62~2.72	2.62~2.72	3.79	—	2.62~2.72	—
4		6.34		2.50~2.66		3.68	—	2.50~2.66	—
5	5.13	6.34		2.5~2.7		3.69		2.5~2.7	
6	5.28	6.43	5.0	—	4.15	3.76	3.72	2.55	—
7	4.91	6.41	5.18	2.24	4.08	3.66	3.78	2.59	—
8	6.14	6.51	5.08	—	3.9	2.2	—	3.02	1.45
8*	6.22	6.41	5.2~5.0	—	5.4~4.8	2.7~1.9	—	3.17	1.57
9	5.75	6.41	5.09	—	3.9	1.99	—	2.58	1.25
9*	6.13	6.38	5.07	—	5.4~4.9	2.5~2.0	—	3.06	1.47
10	6.11	6.53	5.04	—	3.9	2.1	—	2.88	1.45

* data of its actate each other

reactions, it could be postulated that the compound fell in the monoterpene iridoid glycoside.

The glucosyl group must be attached to the hemiacetalic OH at C_1 by analogy of other glycosides of this class. Spectroscopic support of this location is the chemical shift of the anomeric proton at C_1 (6.42ppm) when compared with reported resonce of the same proton of aglycones(8, 14). The signal of the anomeric proton at C'_1 (d. J=ca. 7Hz) proved that glucose is in the β-configuration(13).

Since absorption bands at 1650cm^{-1} and 750 cm^{-1} indicate that the double bond is cis form, methyl group(1380cm^{-1}) and acetyl group(1970 cm^{-1}) can not be linked to the same ring which has the double bond. Thus methyl and acetyl group must be linked to the cyclopentane ring.

In the PMR spectrum of the compound, the doublet-doublet signal from C_4-H suggested the existance of a hydroxyl group at C_5 position (13). This hydroxyl group was determined that it is a tertiary hydroxyl group. The statement can be deduced from the result that the signal at 3.5ppm was markedly reduced when the acetylated compound was treated with D_2O.

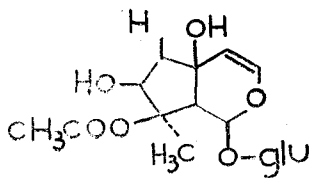
Seven actate signals in the PMR data of the acetylated compound indicate another two secondary hydroxyl groups at cyclopentane ring.

The proton of C_9 must be in the β-configuration by the coupling constant unable to measure between C_1 and C_9(dihedral angle$\simeq$90°). Probaly the cyclopentane and pyran rings have a cis junction and hydroxyl group at C_5 is β-form in accordance with all the known iridoid compounds, and in particular harpagide.

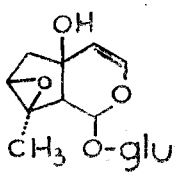
After acetonization, in view of two main spots of products on TLC, hydroxyl groups at C_6 and C_7 can be cis form. That is, all hydroxyl groups at C_5, C_6, and C_7 seem to be in the β-configuration(15).

In view of the disappearance of CH_3 group at C_4 from the IR spectrum and the chemical shift of protons in cyclopentane and pyran ring suggested the presence of both acetyl and methyl group at C_8 position.

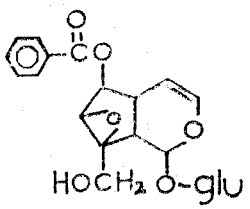
It was established that if iridoid glycosides contained CH_3COO-(α)and CH_3-(β) at the position of C_8, proton signals at C_1 and C_9 showed



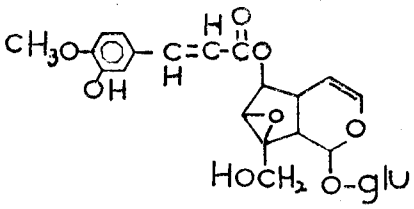
1. Jaranidoside



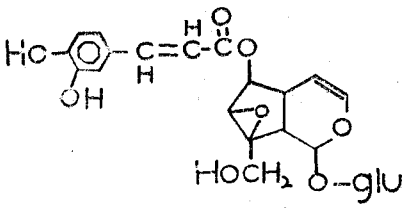
2. Galiridoside



3. Veronicoside

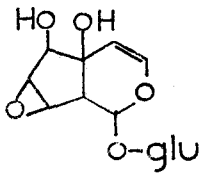


4. Menecoside

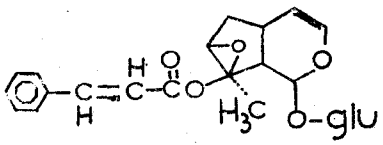


5. Verminoside

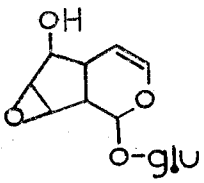
6. Stilbericoside



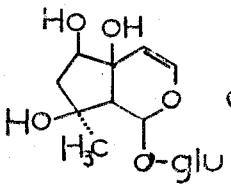
8. Harpagoside



7. Unedoside



9. Harpagide



10. 8-O-acetyl harpagide

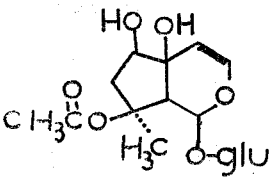


Fig. 7. Structures of Jaranidoside analogs (8, 10-13)

at 5.5-5.6 ppm and 2.3-2.58 ppm, respectivly. But if iridoid glycosides contained CH_3COO-(β) and CH_3-(α) at C_8, proton signals at C_1 and C_9 showed 5.7-5.9 ppm and 2.75-3.24 ppm, respectively. According to this information, the result with the isolating compound showed having CH_3COO-(β) and CH_3-(α) structure. Thus, in view of the results from chemical reactions and instrumental analyses, the compound isolated from Jaran-cho can be 1-(β-glucopyranosyloxy)-5, 6, 7, 8-tetrahydroxy-8-methyl-cyclopenta [c] pyran-8-acetyl ester.

Although physiological activities of the compound could not be studied systematically because of the limited time, it showed that the compound had a stimulating activity on the contraction of smooth and cardiac muscle.

But it was reported that a total extract of

Ajuga spectailisb Nakai had a strong antitumor activity(9). Thus, it can be suggested that further studies on this compound might be shed a light on the development of a new antitumor agent.

Extensive studies on Jaran-cho for the chemistry as well as for the pharmacological action of the components should be carried on for a useful information in both aspects.

References

1. H.K. Wagner, P.M. Wolff: New Natural Products and Plant Drugs with Pharmacological or Therapeutical Activity 145-155 (1977).
2. I. Inagaki: *Phytochemistry*, 117 (1974).
3. T. Sakan et al: *Pippon Kagaku Zasshi*, **90**, 507 (1969).
4. K. Nakanishi, T. Goto, S. Ito, S. Natori S. Nozoe: Natural Products Chemistry, vol. 1. (1975)
5. R. Hegnauer, P. Kooiman: *Planta Medica*, 33, No. 1, 1-33. (1978)
6. T.H. Chung: Korean Flora, 1972.
7. H.S. Ahn, C.Y. Lee:Nomina Plantarum Koreanum
8. H. Lichti, A. von Wartburg: *Helv. Chi. Acta*, **49**, 1552 (1966).
9. B. S. Chung. W.K. Chung, C.H. Kim, M.W. Chun: *J. Pharm. Soc. Kor.*, **14**, 51-53 (1970).
10. O. Sticher: *Helv. Chim. Acta.*, **53**, 2010 (1970).
11. O. Sticher, Fatma U., Afifi-Yazar: *Helv. Chim. Acta.*, **62**, 530, (1979).
12. H. Rimpler, H. Pister: *Z. Naturforsch*, 29c, 368, (1974).
13. M.L. Scarpati, M. Guiso: *Tetrahedron*, **23**, 4709, (1967).
14. H. Wartburg; *Tet. Lett.*, 835, (1964).
15. A. Bianco. M. Guiso, C. Iavorone. C. Trogalo.: *Gazz Chin. Ital.*, 105, (1975).