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# CHEMICAL CONSTITUENTS IN THE GENUS *ACHLYS*

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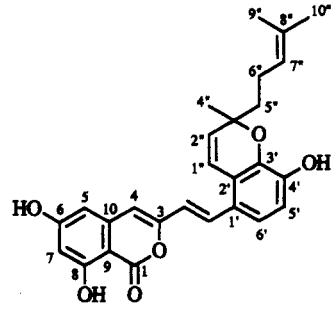
ABSTRACT.—The structure of a new isocoumarin derivative, achlisocoumarin IV [**1**], isolated from the underground parts of *Achlys triphylla* was characterized by means of its spectroscopic properties. The chemical constituents of *Ac. triphylla* and *Ac. triphylla* subsp. *japonica* were compared by hplc to find their chemotaxonomic similarities and differences. Results showed a chemotaxonomically close relationship.

The genus *Achlys* has been classified into the same subtribe, Epimediiae, as *Epimedium, Vancouveria,* and *Jeffersonia (Plagiorhegma)* (1). To elucidate the chemotaxonomic intra- and inter-relationships of the subtribe's genera, we have examined the chemical constituents of *Epimedium* (2–5) and *Vancouveria* (6–9). Isolation and structure determination of isocoumarin derivatives (10) and flavonol and isoflavone glycosides as well as some phenolic compounds (11) in *Achlys triphylla* (Sm.) DC. (Berberidaceae) have also been studied. This paper describes the structure of a new coumarin derivative isolated from the roots of *Ac. triphylla* and the relationship between *Ac. triphylla* and *Ac. triphylla* subsp. *japonica* (Maxim.) Kitam. based on chemical constituents in the underground parts.

## RESULTS AND DISCUSSION

A $CH_2Cl_2$ extract of the underground parts of *Ac. triphylla* was repeatedly subjected to Si gel cc to give compound **1**, genistein, and 3′-methylorobol.

Compound **1** was obtained as a yellow powder and reacted positively to $FeCl_3$ and in Gibbs reagent. The $[M]^+$ at *m/z* 446.1757 in the hreims corresponded to $C_{27}H_{26}O_6$ (calcd 446.1729). In the $^1$H-nmr spectrum, a set of meta-coupled one-proton doublets [δ 6.40 and 6.50 (each $J$=2 Hz)], a broad olefinic one-proton singlet (δ 6.64), two trans-olefinic protons [δ 6.75 and 7.56 (each $J$=16 Hz)], and a chelated hydroxyl group (δ 11.14) were observed. In the nOe experiments, the effects were revealed between the proton at δ 6.64 and one meta-coupled proton (δ 6.50) (20.7%) as well as the trans-olefinic protons (δ 6.75) (23.5%) (Figure 1). These findings indicated the partial structure of **1** was a 5,7-dioxygenated isocoumarin substituted with CH=CH at C-3. In the eims, a fragment ion *m/z* 177 supported the partial structure, drawn as **1a**.



**1**

On the other hand, the $^1$H-nmr spectrum showed the presence of three Me groups (δ 1.42, 1.57, and 1.64), a moiety of $CH_2$-$CH_2$ (δ 1.69 and 2.15), an olefinic methine (δ 5.12), and a chromene ring as a set of cis-olefinic protons [δ 5.92 and 6.91 (each $J$=10 Hz)] in addition to a tetra-substituted benzene ring in a set of one-proton doublets [δ 6.78 and 7.19 (each $J$=8 Hz)]. This indicated that the

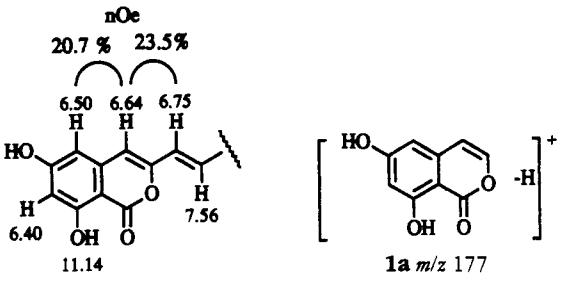



FIGURE 1

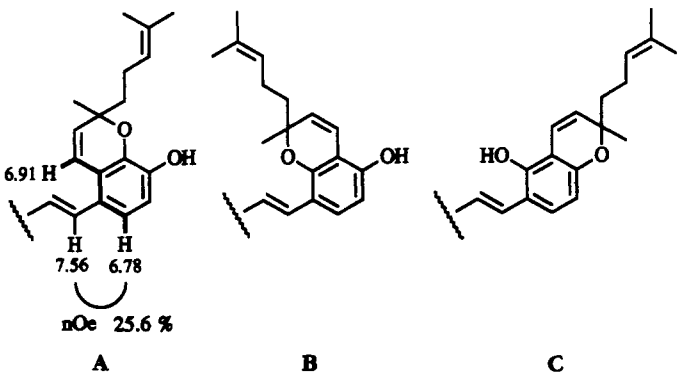
chromene ring substituted with a $C_6H_{11}$ side chain was fused on the tetra-substituted benzene ring. A fragment ion at *m/z* 363, caused by elimination of $C_6H_{11}$ from the parent ion in the eims, supported the presence of the side chain. Therefore, the partial structure would be formed by oxidative cyclization between a $C_{10}$ side chain (geranyl chain) and its adjacent hydroxyl group on the benzene ring. By the differences of oxygenation patterns on the benzene ring (2,4- or 3,4-) and by the directions of cyclization, three possible partial structures are proposed.

Possible partial structures of **1** are shown as **A**, **B**, and **C**. The $^1$H-nmr spectral data presented two possibilities, that is, **A** and **B**. An nOe (25.6%) was observed between the trans-olefinic proton (δ 7.56) and the aromatic proton (δ 6.78), and one of the olefinic protons of chromene ring (δ 6.91) had a long range coupling with the aromatic proton (δ 6.78) through $^5J$. This led to the conclusion that the partial structure was **A**. The whole structure of a new isocoumarin derivative named achlisocoumarin IV, then, was determined to be **1**.

The other two compounds were determined by the spectral analysis to be genistein and 3′-methylorobol.

To clarify the relationship of the chemical constituents between *Ac. triphylla* and *Ac. triphylla* subsp. *japonica*, roots of the latter plant were examined. The roots of *Ac. triphylla* subsp. *japonica*, mentioned later, were subjected to the same methods of extraction and isolation as those of *Ac. triphylla* (10,11). Seven compounds, kaempferol, isorhamnetin, vanillic acid, *p*-hydroxybenzoic acid, achlisocoumarins I (10) and IV, and isorhamnetin 3-*O*-β-D-glucopyranosyl-(1↦3)-β-D-galactopyranoside, were found. Their structures were characterized by analysis of spectral data and comparison with the authentic samples.

Each MeOH extract was checked by hplc to survey quantity and type of chemi-



cal constituents in the roots of both plants. In the roots of *Ac. triphylla*, genistein 7-*O*-glucosyl-(1↦2)-glucoside (Rt 9.5 min), genistin (genistein 7-*O*-glucoside) (13.3 min), isorhamnetin 3-*O*-glucosyl-(1↦6)-galactoside (15.6 min), syringetin 3-*O*-(6-Ac)glucosyl-(1↦6)-galactoside and isorhamnetin 3-*O*-(6-Ac)glucosyl-(1↦6)-galactoside (20.1 min), syringetin 3-*O*-(4,6-diAc)glucosyl-(1↦3)galactoside (24.4 min), achlisocoumarin II (47.6 min), and achlisocoumarin IV (47.9 min) appeared at the times given in parentheses.

On the other hand, in the roots of *Ac. triphylla* subsp. *japonica*, the total contents of genistin or isorhamnetic glycosides with acetyl group(s) were relatively less than those in *Ac. triphylla*, in spite of the presence of achlisocoumarins II and IV. Qualitative similarities of both chemical constituents shown in the hplc chart showed the close relationship between *Ac. triphylla* and *A. triphylla* subsp. *japonica*.

Our previous and present work make clear that isocoumarin derivatives, flavonol glycosides, and isoflavones are important chemical constituents of the genus *Achlys*. To the best of our knowledge, however, 3-phenylethyl isocoumarin derivatives such as achlisocoumarins I–IV have been reported only as constituents of *Agrimonia* (Rosaceae) under the name of agrimonol [6,8-dihydroxy-(4′-methoxyphenethyl)-dihydrocoumarin] (12). The other compounds, such as isoflavones, in the genus *Achlys* are commonly contained in the Rosaceous plants. Generally, the chemical constituents of the genus *Achlys* (Berberidaceae) are similar to those in these plants in the Rosaceae.

## EXPERIMENTAL

PLANT MATERIAL.—Roots of *Ac. triphylla* were collected in July 1991 at Ashland, Oregon, and those of *Ac. triphylla* subsp. *japonica* in August 1990 at Jozankei, Hokkaido, Japan. The vouchers were deposited in the herbarium of Gifu Pharmaceutical University.

EXTRACTION AND ISOLATION.—The dried and ground roots of *Ac. triphylla* (410 g) were extracted with $CH_2Cl_2$ under reflux. After solvent removal, the resulting extract (10 g) was chromatographed on Si gel eluted with increasing concentrations of EtOAc in *n*-hexane. An *n*-hexane–EtOAc (4:1) eluent was rechromatographed by using a solvent of *n*-hexane–EtOAc–MeOH (8:2:1) to produce **1** (10 mg) and a mixture of isoflavones. The mixture was separated again by Si gel chromatography eluted with $CHCl_3$/MeOH to give genistein (2 mg) and 3′-methylorobol (2 mg). The roots (112 g) of *Ac. triphylla* subsp. *japonica* were treated as the same way to give kaempferol (4 mg), isorhamnetin (6 mg), achlisocoumarin I (2 mg), achlisocoumarin IV (2 mg), vanillic acid (3 mg), *p*-hydroxybenzoic acid (2 mg), and isorhamnetin-3-*O*-β-D-glucopyranosyl-(1↦3)-β-D-galactopyranoside (12 mg).

HPLC CONDITIONS.—Pulverized samples (200 mg) were extracted with MeOH (3 ml) under reflux (15 min×3). After centrifugation, each supernatant was combined, MeOH added to make up to 10 ml, and then subjected to reversed-phase hplc. Hplc analysis was carried out on Capcellpack $C_{18}$ AG-120A (Shiseido, Japan), MeCN/$H_2O$ gradient of 15–40% MeCN in 20 min, then to 100% MeCN in another 10 min at a flow rate of 1 ml/min with detection at 272 nm. Column temperature was 40°.

*Achlisocoumarin IV* [**1**].—A yellow powder: hreims *m/z* 446.1757 (calcd 446.1729 for $C_{27}H_{26}O_6$); eims *m/z* (rel. int.) *m/z* 446 (40), 363 (100), 177 (16), 121 (15), 109 (12); $^1$H nmr ($Me_2CO$-$d_6$) δ 1.42, 1.57, 1.64 (3H each s, Me-4″, Me-9″, and Me-10″), 1.69, 2.15 (2H each, m, H-5″ and H-6″), 5.12 (1H, m, H-7″), 5.92 (1H, d, *J*=10 Hz, H-2″), 6.40 (1H, d, *J*=2 Hz, H-7), 6.50 (1H, br d, *J*=2 Hz, H-5), 6.64 (1H, s, H-4), 6.75 (1H, br d, *J*=16 Hz, H-8′), 6.78 (1H, br d, *J*=8 Hz, H-6′), 6.91 (1H, br d, *J*=10 Hz, H-1″), 7.19 (1H, d, *J*=8 Hz, H-5′), 7.56 (1H, d, *J*=16 Hz, H-7′), 7.93, 9.78 (1H each, br s, OH), 11.14 (1H, s, 8-OH); uv λ max (MeOH) 249, 258 sh, 265 sh, 284 sh, 345 sh, 369 nm, (+NaOMe) 270, 300 sh, 411.

## ACKNOWLEDGMENTS

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