NOVEL ISOCOUMARIN DERIVATIVES FROM ACHLYS TRIPHYLLA (BERBERIDACEAE)

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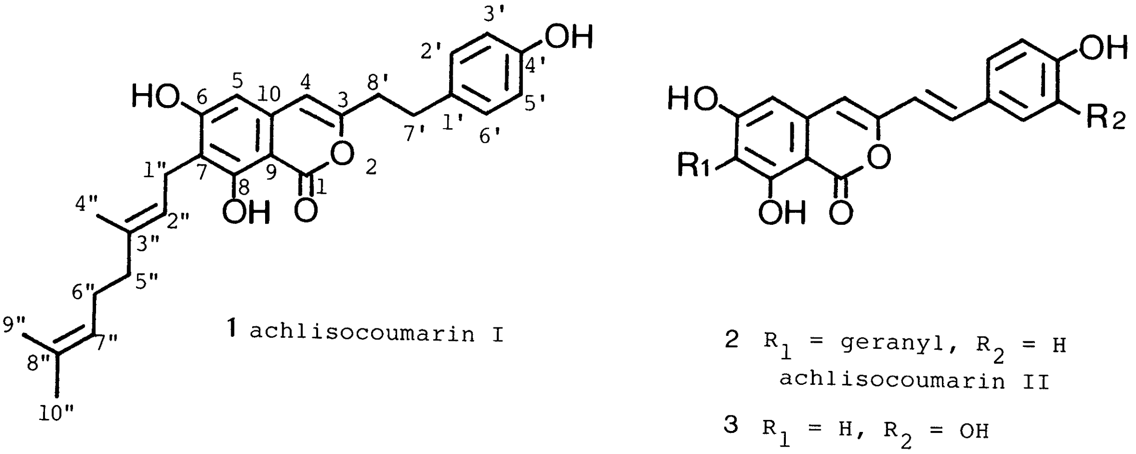
Three new phenolic compounds with a novel skeleton were isolated from the underground parts of *Achlys triphylla* (Berberidaceae). The structures were established by means of spectroscopic methods to be 7-geranyl-6,8-dihydroxy-3-(4'-hydroxyphenylethyl)isocoumarin (1) for achlisocoumarin I, 7-geranyl-6,8-dihydroxy-3-(4'-hydroxyphenylethenyl)isocoumarin (2) for achlisocoumarin II, and 6,8-dihydroxy-3-(3',4'-dihydroxyphenylethenyl)isocoumarin (3) for achlisocoumarin III, respectively.

KEYWORDS *Achlys triphylla*; Berberidaceae; 7-geranyl-6,8-dihydroxy-3-(4'-hydroxyphenylethyl)isocoumarin; 7-geranyl-6,8-dihydroxy-3-(4'-hydroxyphenylethenyl)isocoumarin; 6,8-dihydroxy-3-(3',4'-dihydroxyphenylethenyl)isocoumarin

Except for an old report[1)] suggesting the occurrence of coumarin, the chemical constituents of *Achlys triphylla* (J. E. Sm.) DC have not been carefully investigated. We are interested in the chemical constituents of the plant to determine its chemotaxonomic relationships with other members of the subtribe Epimedinae, and to look for chemicals with medicinal properties.

The plant material used in this experiment was collected in July 1988 near Ashland, Oregon, U.S.A. The



1 achlisocoumarin I

2 R_1 = geranyl, R_2 = H
achlisocoumarin II

3 R_1 = H, R_2 = OH
achlisocoumarin III

Chart 1

dried and pulverized underground parts of A. triphylla were extracted with methanol. An ethyl acetate-soluble layer of the methanol extract was separated with silica gel chromatography eluted with C_6H_6-AcOEt to yield vanillic acid, p-hydroxybenzoic acid, and compounds **1**-**3**.

Compound **1**,$^{2)}$ achlisocoumarin I, was obtained as a yellow powder. It positively reacted in both $FeCl_3$ and Gibbs tests. Because of the high-resolution MS (M^+ : m/z 434.2078), its molecular formula is thought to be $C_{27}H_{30}O_5$ (Calcd. 434.2093). In addition to three hydroxyl groups observed at δ 11.49 (chelated), 9.63 and 8.15,$^{3)}$ the presence of a geranyl [(E)-3,7-dimethyl-2,6-octadienyl] or neryl [(Z)-3,7-dimethyl-2,6-octadienyl] (C_{10} unit) was suggested by the appearance in the ^{1}H-NMR spectrum of three three-proton singlets at 1.55, 1.60 and 1.79, a four-proton multiplet at 2.05, and a one-proton multiplet at 5.06 and 5.27. A 4-hydroxyphenylethyl moiety (C_6-C_2 unit) was also suggested by two two-proton triplets (J= 7 Hz) at δ 2.80 and 2.98 assignable to ϕ-CH_2-CH_2-, and two two-proton doublets (J= 8 Hz) at δ 6.74 and 7.08 to 1,4-disubstituted benzene. A geranyl group is preferable to a neryl in the C_{10} moiety after comparison of the chemical shifts at C-4" and C-5" with those of the authentic compound$^{4)}$ in the ^{13}C-NMR spectra. From the three possible partial structures (**A**-**C**) shown in Chart 2, the remaining skeleton, composed of a C_6-C_3 unit, was regarded as an isocoumarin ring (1H-2-benzopyran-1-one). This decision was based on the following observations: (a) absorption bands at 1670 cm^{-1} due to a lactone ring in the IR, and at 250, 280, 293sh, 333 and 380sh in the UV$^{5)}$ spectra; (b) the presence of a chelated hydroxyl group (δ 11.49); (c) an NOE effect between a singlet (δ 6.29)reasonably assigned to an olefinic proton at C-4 and a proton at δ 6.44; (d) NOE effects between the olefinic proton and one of methylenes at C-8' (δ 2.80); and (e) proton-proton long range coupling in the 2D-NMR between the olefinic proton at C-4 and the methylene at C-8', and between the other methylene at C-7' (δ 2.98) and the protons at C-2' and C-6' (Chart 3: solid line; NOE effect, broken line; H-H long range coupling). Therefore, the partial structure of **1** is now thought to be an isocoumarin derivative with p-hydroxyphenylethyl group substituted at its C-3. The position of the geranyl group, that is, whether the ring A has 7-geranyl-6,8-dihydroxy or 6-geranyl-7,8-dihydroxy group, was determined by the ^{1}H-^{13}C long range COSY caused by cross peaks between a proton at C-1" in the geranyl group and carbons at C-6 and C-8. Hence, the structure of **1** was concluded to be 7-geranyl-6,8-dihydroxy-3-(4'-hydroxyphenylethyl)isocoumarin.

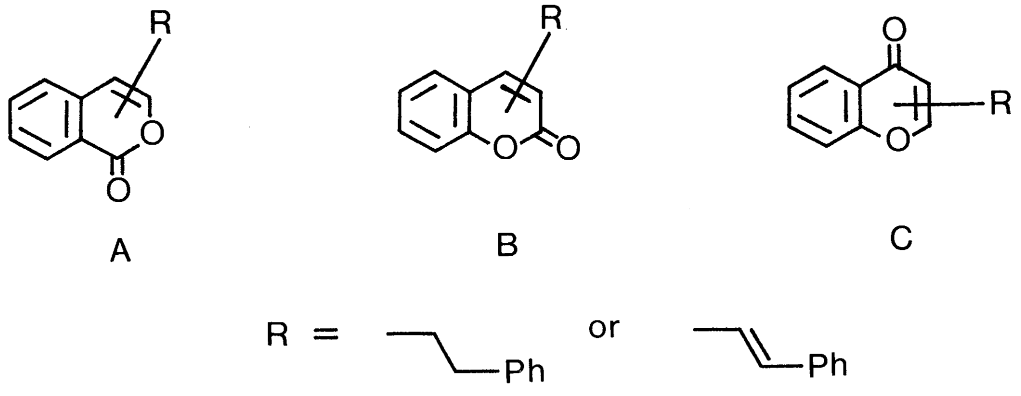


Chart 2

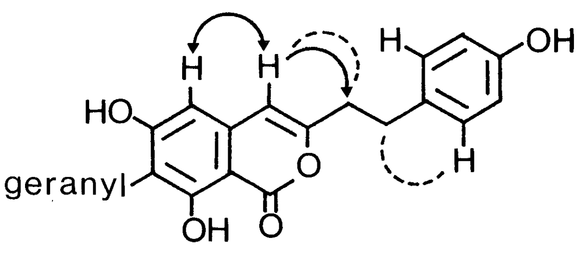


Chart 3

Compound **2**,$^{6)}$ achlisocoumarin II, was obtained as a yellow powder. The high-resolution MS (M^+ : 432.1958) determined its empirical formula to be $C_{27}H_{28}O_5$ (Calcd.

432.1936), two hydrogens less than compound **1**. In the ^{1}H-NMR spectrum, two one-proton doublets (*J*= 16 Hz) at δ 6.76 and 7.26 were observed instead of two two-proton triplets in **1**. These spectral data indicated that **2** is an isocoumarin derivative with a *p*-hydroxyphenylethenyl group at C-3. Therefore, **2** was characterized as 7-geranyl-6,8-dihydroxy-3-(4'-hydroxyphenylethenyl)isocoumarin.

Compound **3**,[7] achlisocoumarin III, was also obtained as a yellow powder. By its empirical formula $C_{17}H_{12}O_6$ (Calcd. 312.0634) confirmed by the high-resolution MS (M^+ : 312.0643) and the ^{1}H-NMR spectrum (no appearance of methyl signals due to a geranyl group), **3** lacked the geranyl group. Two *meta*-coupled protons (*J*= 2 Hz) at δ 6.37 and 6.45 were assignable to H-7 and H-5. NOE effects were observed between H-4 and H-5, and between H-4 and H-8'. Therefore, the partial structure of **3** was established as 6,8-dihydroxyisocoumarin with a phenylethenyl moiety at C-3. The positions of two hydroxyl groups on a side-phenyl moiety was determined to be at C-3' and C-4' based on the following evidence: a double-doublet *ortho*- and *meta*-coupled at δ 7.03 assignable to H-6' showed an NOE effect with H-7', which eliminated the other possible substitution patterns such as 2',4'- and 2',5'-dioxygenation. Hence, the structure of **3** was concluded to be 6,8-dihydroxy-3-(3',4'-dihydroxyphenylethenyl)isocoumarin.

REFERENCES AND NOTES

1) C.E. Bradley, J. Am. Chem. Soc., 29, 606 (1907).

2) **1**: ^{1}H-NMR (270 MHz, acetone-*d*$_6$) δ : 1.55 (3H, br s, H-9"), 1.60 (3H, d, *J*= 1 Hz, H-10"), 1.79 (3H, d, *J*= 1 Hz, H-4"), 2.05 (4H, m, H-5",6"), 2.80 (2H, t, *J*= 7 Hz, H-8'), 2.98 (2H, t, *J*= 7 Hz, H-7'), 3.38 (2H, d, *J*= 7 Hz, H-1"), 5.06 (1H, m, H-7"), 5.27 (1H, m, H-2"), 6.29 (1H, s, H-4), 6.44 (1H, s, H-5), 6.74 (2H, d, *J*= 8 Hz, H-3',5'), 7.08 (2H, d, *J*= 8 Hz, H-2',6'), 8.15, 9.63, 11.49 (1H, each s, OH). ^{13}C-NMR (67.5 MHz, acetone-*d*$_6$) δ : 16.8 (C-4"), 18.2 (C-9"), 23.0 (C-1"), 26.3 (C-10"), 27.9 (C-6"), 33.4 (C-7'), 36.5 (C-8'), 41.0 (C-5"), 100.2 (C-9), 103.2 (C-5), 105.5 (C-4), 115.7 (C-7), 116.6 (C-3',5'), 123.3 (C-2"), 125.6 (C-7"), 130.7 (C-2',6'), 132.1, 132.6 (C-1',C-8"), 136.1 (C-3"), 138.4 (C-10), 157.2, 157.6 (C-3, C-4'), 162.2, 164.3 (C-6, C-8), 168.0 (C-1).

3) When $AlCl_3$ was added, a drastic bathochromic shift (35 nm) occurred in the UV spectrum.

4) T. Fukai, T. Fujimoto, Y. Hano, T. Nomura, and J. Uzawa, Heterocycles, 22, 2805 (1984); M. Kozawa, N. Morita, K. Baba, and K. Hata, Yakugaku Zasshi, 98, 210 (1978).

5) A.S. Feliciano, J.M. Corral, L.M. Canedo, and M. Medarde, Phytochemistry, 29, 945 (1990).

6) **2**: ^{1}H-NMR (270 MHz, acetone-*d*$_6$) δ : 1.55, 1.60 (3H, br s, H-9",10"), 1.80 (3H, s, H-4"), 2.05 (4H, m, H-5",6"), 3.41 (2H, d, *J*= 7 Hz, H-1"), 5.08 (1H, m, H-7"), 5.29 (1H, m, H-2"), 6.55, 6.56 (1H, each s, H-4,5), 6.76 (1H, d, *J*= 16 Hz, H-7'), 6.89 (2H, d, *J*= 8 Hz, H-3',5'), 7.26 (1H, d, *J*= 16 Hz, H-8'), 7.52 (2H, d, *J*= 8 Hz, H-2',6'), 11.48 (1H, s, OH).

7) **3**: ^{1}H-NMR (270 MHz, acetone-*d*$_6$) δ : 6.37 (1H, d, *J*= 2 Hz, H-7), 6.45 (1H, d, *J*= 2 Hz, H-5), 6.55 (1H, s, H-4), 6.72 (1H, d, *J*= 16 Hz, H-7'), 6.86 (1H, d, *J*= 8 Hz, H-5'), 7.03 (1H, dd, *J*= 2, 8 Hz, H-6'), 7.15 (1H, d, *J*= 2 Hz, H-2'), 7.21 (1H, d, *J*= 16 Hz, H-8'), 8.12, 8.75, 11.14 (1H, each s, OH). ^{13}C-NMR (67.5 MHz, acetone-*d*$_6$) δ : 100.0 (C-9), 103.2 (C-7), 104.8 (C-5), 106.8 (C-4), 114.9 (C-6'), 116.9 (C-5'), 118.0 (C-8'), 121.7 (C-2'), 130.0 (C-1'), 134.1 (C-7'), 141.4 (C-10), 147.0, 148.2 (C-3',4'), 154.1 (C-3), 165.2 (C-8), 166.8 (C-6), 167.6 (C-1).

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