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KONYANIN: A NEW LIGNAN FROM HAPLOPHYLLUM VULCANICUM

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Abstract: Konyanin (1), obtained from Haplophyllum vulcanicum (Rutaceae), is the first known 1,4-dihydroarylnaphthalene lignan in which the lactone carbonyl is bonded to C-3 rather than to C-2. Detailed NMR spectral data have been compiled for the accompanying lignans (-)-kusunokinin (5) and diphyllin (7).

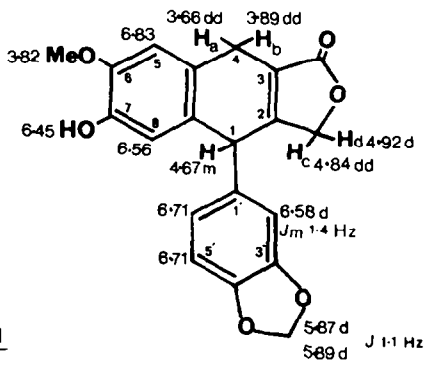
The genus Haplophyllum A. Juss. (Rutaceae) comprises about 70 perennial herbs spread out from the Mediterranean to eastern Siberia. It is known for elaborating various quinoline alkaloids,[1] as well as amides,[1] coumarins[2,3] and lignans.[4-7] A literature survey indicated that less than half of the species of this genus had been investigated, even though Haplophyllum alkaloids exhibit a number of interesting pharmacological effects, viz. CNS depressant, sedative, analgesic, anticonvulsive, hypothermic, cytotoxic and estrogenic. These facts induced us to study the natural products present in the hitherto uninvestigated H. vulcanicum Boiss. et Heldr. collected east of Konya, in the central Anatolian plateau.

We now report on the contents of the so-called "non-basic" fraction of this plant. The new lignan konyanin (1) was obtained, together with the known lignans (-)-kusunokinin (5) and diphyllin (7), the widespread coumarin scopoletin (≡ 6-methoxy-7-hydroxycoumarin), and the furoquinoline alkaloid kokusaginine (≡ 6,7-dimethoxyfuroquinoline). Whereas the presence of diphyllin (7) appears to be ubiquitous in the Haplophyllums, this is the first report of the isolation of kusunokinin (5) from this genus.

The new lignan konyanin (1) crystallized from methanol as colorless needles, $C_{20}H_{16}O_6$. A flat CD curve indicated the compound to be inactive. The IR spectrum showed hydroxyl and carbonyl absorptions at 3540 and 1732 cm^{-1}, respectively. The UV spectrum exhibited λ max (MeOH) 289 (log ε 3.82) with an inflection at 231 nm (log ε 3.99). Consonant with the presence of a phenolic function, the spectrum underwent a bathochromic shift upon addition of alkali. Additionally, konyanin readily furnished O-acetyl (2) as well as O-methyl (3) derivatives.

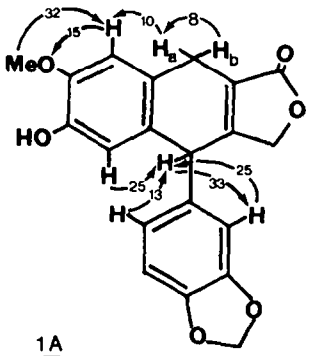
A special effort was made to obtain a detailed ^1H NMR spectrum for konyanin (1). All sixteen protons are clearly visible in the spectrum which was obtained at 360 MHz in CD_3CN solution, and which has been summarized in expression 1. The presence of methoxyl and methylenedioxy substituents are clearly discernable. Of the five aromatic protons, two are as singlets (δ 6.56 and 6.83), while the remaining three span the 2', 5', and 6' positions of the lower pendant phenyl ring. Significantly, the H-8 absorption (δ 6.56) for konyanin underwent a significant downfield shift to δ 6.84 upon acetylation, as indicated in expression 2.

The five aliphatic protons of konyanin (1) presented a complex picture in the NMR spectrum which may be summarized as follows. The C-4 non-equivalent methylene protons at δ 3.66 and 3.89 are split not only by each other (J_{gem} = 23.0 Hz), but also by long range coupling with H-1

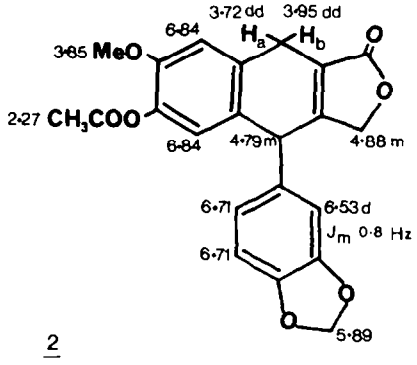


1

$J_{4a,4b}$ 23·0 Hz
$J_{4a,1}$ 4·3 Hz
$J_{4b,1}$ 4·2 Hz
$J_{Hc,Hd}$ 17·1 Hz
$J_{Hc,1}$ 2·4 Hz



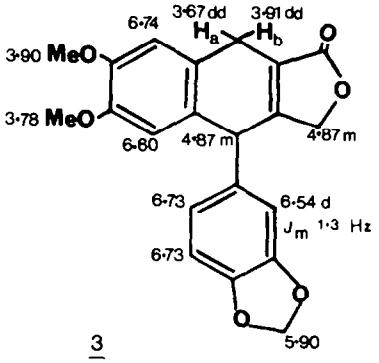
1A



2

$J_{4a,4b}$ 22·6 Hz
$J_{4a,1}$ 3·9 Hz
$J_{4b,1}$ 4·4 Hz

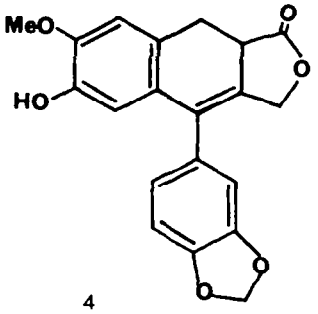
NMR spectrum in $CDCl_3$ at 200 MHz



3

$J_{4a,4b}$ 22·3 Hz
$J_{4a,1}$ 4·2 Hz
$J_{4b,1}$ 4·1 Hz

NMR spectrum in $CDCl_3$ at 200 MHz



4

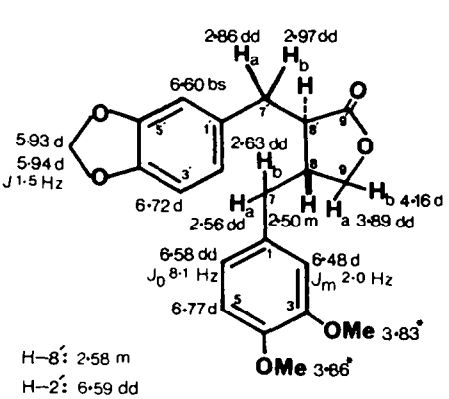
which appears as a multiplet at δ 4.67. In turn, H-1 is also coupled to one of the two methylene protons incorporated in the γ-lactone ring: These methylene protons fall at δ 4.84 and 4.92 (J_{gem} = 17.1 Hz), and it is the upfield δ 4.84 absorption which is further split by long range coupling with H-1. The proper spin decoupling experiments were run to confirm each of the assignments.

A proton-proton differential NOE study was also carried out which gave unequivocal support to the structural assignment for konyanin (1). The results are given in expression 1A. The H-1 absorption (δ 4.67) exhibits strong reciprocating NOE with H-2' (δ 6.58) as well as with H-6' (δ 6.71). This finding rules out alternate structure 4 for konyanin. Additionally, irradiation of the H-4 absorption at δ 3.66 resulted in enhancement of the H-5 singlet absorption at δ 6.83. This same singlet absorption was also substantially enhanced by irradiation of the methoxyl singlet at δ 3.82. It follows that the methoxyl substituent is located at C-6 rather than at the alternate C-7 site.

The mass spectrum of konyanin (1) shows a molecular ion peak m/z 352. There is also an important peak m/z 230 due to loss of the methylenedioxybenzene moiety from the molecular ion.

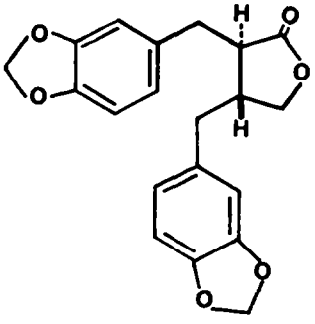
A significant observation concerning konyanin (1) is that it is the first example of a 1,4-dihydroarylnaphthalene lignan in which the lactone carbonyl is bonded to C-3 rather than to C-2.

The second lignan isolated was identified as the known (-)-kusunokinin (5). This compound was first obtained from Cinnamomum camphora Sieb. (Lauraceae) in 1975.[8] But quite recently it has been claimed unjustifiably as a new lignan from Virola sebifera Aubl. (Myristicaceae).[9] Since none of the earlier investigators had assigned specific ^{1}H NMR resonances, we have summarized the high resolution proton spectrum (360 MHz, $CDCl_3$) around expression 5, with the assignments again supported by spin decoupling experiments.[10,11]



5

$J_{7a,7b}$ 11·7 Hz
$J_{7a,8}$ 7·6 Hz
$J_{7b,8}$ 4·7 Hz
$J_{7a,7b}$ 14·0 Hz
$J_{7a,8}$ 7·0 Hz
$J_{7b,8}$ 5·1 Hz
$J_{9a,9b}$ 9·3 Hz
$J_{9a,8}$ 6·0 Hz
$J_{9b,8}$ 6·9 Hz

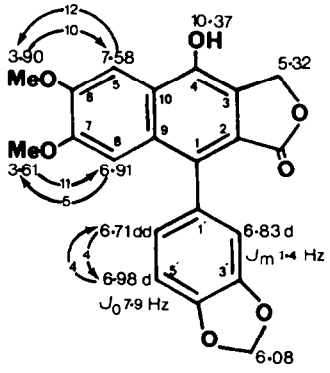


6

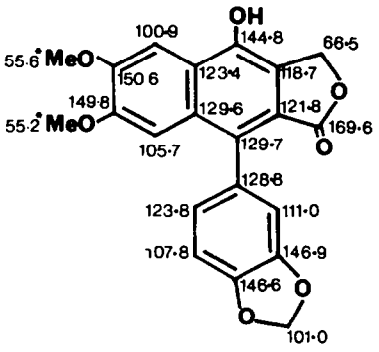
The third lignan from H. vulcanicum was the known diphyllin (7).[12,13] Both the ^{1}H and ^{13}C NMR spectra for this monophenol have been described in a recent report,[10] although some of the assignments were incomplete. We report herewith a detailed description of the ^{1}H and ^{13}C resonances which could prove useful in the structural assignments of related lignans. The DMSO-d_6 ^{1}H spectrum has been summarized around expression 7, in which the NOE values are also quoted. The $CDCl_3$ ^{1}H spectrum (360 MHz) of O-acetyldiphyllin (8) is described in expression 8.

The ^{13}C NMR shift assignments for diphyllin are given around expression 7A, while the multiplicities and the ^{1}J and ^{3}J coupling constants (in Hz) are quoted around expression 7B. The resonances of the protonated carbons, as well as those due to carbons separated from protons by three bonds were identified by selective decoupling of the relevant protons, and observation of the changes in the fully coupled carbon spectrum. Thus, low power decoupling of the proton resonance at δ 6.91 (H-8) for diphyllin collapsed the doublet carbon resonance at δ 105.7

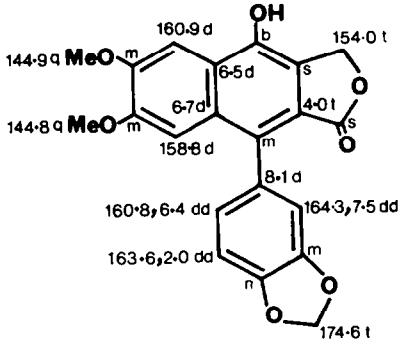
(^1J = 158.8 Hz) to a singlet indicating its association with C-8. Other changes brought by irradiation of H-8 included collapse of the doublet C-10 resonance at δ 123.4 (^3J = 6.5 Hz) into a singlet, along with a sharpening of the multiplet resonance at δ 150.6 and 129.7 related to C-6 and C-1, respectively. In like fashion, decoupling of the H-5 resonance at δ 7.58 allowed us to locate C-4, C-7 and C-9, decoupling of H-5' (δ 6.98) affected the C-1' and C-3' resonances, and irradiation of H-2' (δ 6.83) simplified the C-1 resonance.



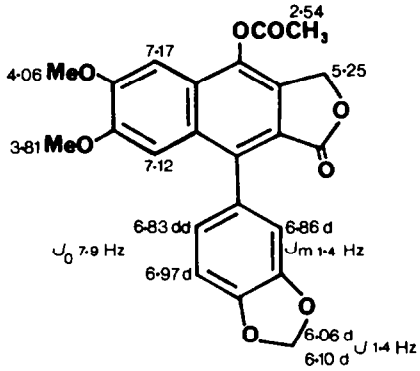
7



7A



7B



8

In an earlier report, the C-4 NMR absorption in diphyllin (7) was assigned to one of the signals in the δ 118.76-129.66 region, while C-1' was related to one of the resonances in the δ 145.04-146.97 range.[10] These assignments are too much upfield in the former case, and too downfield in the latter, and have now been reversed. Additionally, the C-8 and C-2' resonances are presently related to the absorptions at δ 105.7 and 111.0, respectively; in contrast to the earlier assignments of the peaks at δ 111.04 (or 100.99) and 105.78 (or 107.73), respectively.

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Experimental

Plant Collection: The plant (4 kg) was picked in Ulukişla, Niğde, east of Konya, at an altitude of 1450 m, in early July, 1982.[14] Its identity was confirmed by Professor Asuman Baytop of the Faculty of Pharmacy, Istanbul University. A voucher specimen, No. 634, has been deposited in the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Ege University, Izmir.

Isolation: The air-dried powdered aerial parts of the plant were extracted with ethanol (75 L) at room temp. The solvent was evaporated and the residue (≃ 400 g) treated with 5% HCl. The mixture was filtered to remove insoluble materials. The acidic extract was shaken with $CHCl_3$

to remove non-basic constituents (≈ 4.5 g). The aqueous layer upon basification with NH_4OH and extraction with $CHCl_3$ gave an alkaloidal fraction (≈ 3.3 g). The non-basic residue was chromatographed on a column prepared by using 380 g Merck Silica Gel 60H, employing $CHCl_3$-MeOH (98:2) as eluent. Fractions of 50 mL each were collected. The residue (44 mg) from fractions 8-10 was subjected to tlc on silica gel using hexane-ethyl acetate (7:3) to afford kusunokinin (5) (4 mg). The residue (244 mg) from fractions 17-19 was recrystallized from $CHCl_3$-MeOH to afford kokusaginine (120 mg) as fine yellowish white needles. Crystallization of the residue from fractions 26-29 using MeOH gave white crystals of konyanin (1) (12 mg). Fractions 32-36 upon crystallization from MeOH followed by tlc on silica gel using chloroform-methanol-ammonium hydroxide (95:5:0.5) furnished scopoletin (11 mg). Fractions 44-47 provided diphyllin (7) (400 mg) upon crystallization from MeOH.

Kusunokinin (5): Viscous mass; $[\alpha]_D^{25}$ -34.5° ($CHCl_3$, c 0.52); λ max (MeOH) (log ε) 232, 282 sh, 285, 395 sh nm (3.85, 3.64, 3.65, 3.35); ν max ($CHCl_3$) 1765, 1602, 1588, 1500 cm^{-1}; MS m/z (%) 370 (M^+, 44), 192 (4), 178 (11), 177 (16), 151 (82), 135 (100); CD (MeOH) Δε max (nm) -0.6 (288), -3.18 (232).

Kokusaginine: Fine yellowish-white needles ($CHCl_3$-MeOH); m.p. 172° C (lit.[15] m.p. 170-171° C); λ max (MeOH) (log ε) 214, 244, 250, 308, 321, 334 nm (4.21, 4.76, 4.75, 4.09, 4.09, 3.95); λ max (MeOH-HCl) (log ε) 215, 250, 366 nm (4.30, 4.67, 4.22); ν max ($CHCl_3$) 1620, 1587, 1500, 1478, 903 cm^{-1}.

Konyanin (1): Colorless needles from MeOH, m.p. 263-265° C; λ max (MeOH) (log ε) 231 sh, 289 nm (3.99, 3.82); λ max (MeOH-NaOH) (log ε) 239 sh, 292 nm (4.01, 3.88); ν max (KBr) 3540, 2960, 2935, 2895, 1732, 1690, 1585, 1500, 1482, 1465, 1442, 1412, 1355, 1340, 1320, 1278, 1220, 1192, 1152, 1092, 1080, 1017, 980, 918, 900, 858, 840, 800, 790, 780, 748 cm^{-1}; MS m/z (%) 352 (M^+, 100), 308 (10), 307 (30), 291 (7), 277 (16), 263 (8), 249 (5), 230 (16), 201 (17), 187 (16), 176 (7), 165 (8), 152 (35), 122 (33).

Scopoletin: λ max (MeOH) (log ε) 228, 252, 297, 394 nm (4.00, 3.54, 3.56, 3.92); λ max (MeOH-NaOH) (log ε) 212, 239, 392 nm (4.46, 3.83, 4.15); ν max ($CHCl_3$) 3530, 1720 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 3.96 (3H, s, OCH_3), 6.15 (1H, s, OH), 6.27 (1H, d, J = 9.5 Hz, H-3), 6.85 (1H, s, H-8), 6.92 (1H, s, H-5), 7.60 (1H, d, J = 9.5 Hz, H-4); MS m/z (%) 192 (M^+, 100), 177 (61), 164 (27), 149 (47), 121 (22).

Diphyllin (7): Colorless granular crystals, m.p. 285-288° C (lit.[10] m.p. 291° C); λ max (MeOH) (log ε) 229, 267, 308, 321, 357 nm (4.42, 4.63, 3.96, 3.94, 3.71); λ max (MeOH-NaOH) (log ε) 229, 278, 323, 384 nm (4.47, 4.50, 3.73, 3.87); ν max (KBr) 3200, 3000, 2930, 2880, 1715, 1610, 1598, 1480, 1455, 1428, 1392, 1345, 1325, 1250, 1220, 1200, 1160, 1150, 1115, 1095, 1060, 1010, 980, 905, 830, 788, 770, 740, 690, 625 cm^{-1}; ^{13}C NMR (DMSO-d_6, 90 MHz) (multiplicities by SFORD) δ 55.2 (q), 55.6 (q), 66.5 (t), 100.9 (d), 101.0 (t), 105.7 (d), 107.8 (d), 111.0 (d), 118.7 (s), 121.8 (s), 123.4 (s), 123.8 (d), 128.8 (s), 129.6 (s), 129.7 (s), 144.8 (s), 146.6 (s), 146.9 (s), 149.8 (s), 150.6 (s), 169.6 (s); high resolution MS m/z (%) 380.0897 (M^+, $C_{21}H_{16}O_7$, 100) (calc'd 380.0898), 379.0610 ($C_{21}H_{15}O_7$, 15), 352.0933 ($C_{20}H_{16}O_6$, 4), 351.0824 ($C_{20}H_{15}O_6$, 15), 349.0696 ($C_{20}H_{13}O_6$, 5), 335.0584 ($C_{19}H_{11}O_6$, 20), 321.0142 ($C_{18}H_9O_6$, 29).

O-Methylkonyanin (3): Konyanin (2 mg) suspended in acetone (3 mL) was refluxed with dimethyl sulfate (1 drop) and anhydrous potassium carbonate (200 mg) for 12 h. The cooled organic layer was filtered and evaporated to dryness. The residue dissolved in chloroform was passed through a short column of silica gel 1 cm in length. Subsequent evaporation of the organic layer supplied the methyl ether, $C_{21}H_{18}O_6$; ν max ($CHCl_3$) 1755, 1720, 1690, 1610, 1500 cm^{-1}; MS m/z (%) 366 (M^+, 14), 364 (100), 335 (10), 321 (4), 319 (7), 305 (11), 291 (7), 277 (13), 262 (2), 247 (3), 244 (3), 219 (2), 206 (4), 167 (13), 149 (31), 123 (5), 122 (3).

O-Acetylkonyanin (2): Konyanin (2 mg) was warmed with acetic anhydride (4 drops) and fused sodium acetate (5 mg), and then kept overnight. Treatment of the mixture with water and extraction with chloroform eventually furnished the acetate, $C_{22}H_{18}O_7$, ν max ($CHCl_3$) 1755, 1690, 1610, 1480 cm^{-1}; MS m/z (%) 394 (M^+, 10), 352 (15), 350 (7), 307 (4), 291 (1), 279 (2), 259 (3), 230 (3), 206 (28), 185 (7), 149 (100), 132 (12), 123 (13), 122 (12).

O-Acetyldiphyllin (8): Diphyllin (10 mg) dissolved in pyridine (6 drops) was treated with acetic anhydride (12 drops) for 48 h. Work-up gave the acetate, $C_{23}H_{18}O_8$, ν max ($CHCl_3$) 1765, 1620, 1595, 1495, 1475 cm^{-1}, m.p. 234-235° C ($CHCl_3$-hexane) (lit.[12] m.p. 235° C).

References and Footnotes

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