Journal of Natural Products
Vol. 52, No. 4, pp. 749-752, Jul-Aug 1989

PSORACINOL, A NEW LUPANE-TYPE TRITERPENE FROM *PSORALEA PLICATA*

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ABSTRACT.—Psoracinol, a new triterpene of the lupane series, has been isolated from *Psoralea plicata*. Its structure has been elucidated as lup-20(29)-en-7β-ol through chemical and spectroscopic studies.

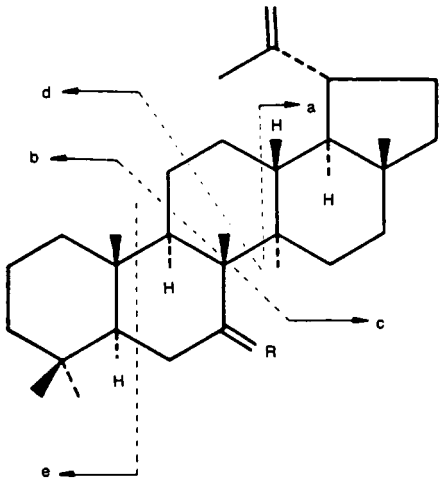
Various species of the genus *Psoralea* (Leguminosae) are reputed in indigenous medicine as anthelminthic, diuretic, diaphoretic, and useful in bilious affections, febrile conditions, and leprosy (1). This has prompted us to carry out systematic studies on the chemical constituents of *Psoralea plicata* Del., a shrub found commonly in the Sind and Punjab areas of Pakistan. Only two coumarins, namely angelicin and psoralen, have so far been reported from this plant (2). As a result of studies on the freshly collected total plant material, we have isolated angelicin, psoralen, and a new pentacyclic triterpene of the lupane series named psoracinol. Herein we report the structural elucidation of this new compound through chemical and spectroscopic studies including 2D-nmr and nOe difference measurements.

RESULTS AND DISCUSSION

Psoracinol [**1**] was isolated from the nonsaponifiable residue of the hexane-soluble fraction as described in the Experimental section. After repeated crystallization from C_6H_6/MeOH, it melted at 192° and showed $[\alpha]_D$ + 19° (c = 0.296, $CHCl_3$). The hrms gave a molecular ion peak at 426.3833 corresponding to the molecular formula $C_{30}H_{50}O$ (calcd 426.3860), indicating six double-bond equivalents in the molecule. The molecular ion peak was also confirmed by fdms. The compound gave a positive Liebermann-Burchard test and a violet coloration with ceric sulfate, suggesting its triterpenic nature. The ir spectrum ($CHCl_3$) showed absorption bands for a hydroxyl group (3400 cm^{-1}) and a terminal methylene (3070, 1640, and 880 cm^{-1}). Its ^{1}H-nmr (300 MHz) spectrum showed signals for seven methyl groups as singlets at δ 0.84, 0.81, 0.78, 0.96, 1.02, 1.06, and 1.67 (3H each). Furthermore, vinylic protons appeared as a pair of broad singlets at δ 4.56 and 4.68 (1H each), while another one-proton double doublet at δ 3.79 could be attributed to a carbinylic proton. The ^{13}C-nmr (75.3 MHz) showed the presence of 30 carbon atoms; their multiplicity assignments were determined by carrying out multipulse ID DEPT experiments using last pulse angles θ = 45°, 90°, and 135°, which revealed the presence of 7 methyl, 11 methylene, and 6 methine carbons.

The secondary nature of the hydroxyl group in **1** followed from its oxidation to a ketone **2**. The latter gave a negative Zimmermann test suggesting the absence of an oxo group at position 3. Compound **2** could be reduced to the parent alcohol on refluxing with $NaBH_4$ for 36 h, indicating the equatorial configuration of the hydroxyl group in **1**.

^{1}H-Nmr and mass spectral evidence revealed the presence of a lupane skeleton in psoracinol. The two 1H broad singlets at δ 4.56 and 4.68 and a 3H singlet at δ 1.67 in ^{1}H-nmr indicated the presence of a lup-20(29)ene system in **1** (3). Mass spectrometric fragmentation is shown in Figure 1. The major fragments at *m/z* 220 (ion d) and 207 (ion b) were due to cleavages across ring C (hrms 220.1831, corresponding to $C_{15}H_{24}O$, and 207.1751, corresponding to $C_{14}H_{23}O$). This suggested that the hy-



1 R=αH, βOH
2 R=O

FIGURE 1. Mass spectrometric fragmentation.

droxyl group was located in either ring A or B (4). This was further substantiated by the occurrence of nonoxygenated fragments involving rings D and E at *m/z* 217 (ion c) and 189 (ion a), respectively (4). Another ion peak at *m/z* 123, ion e (hrms 123.1175, corresponding to C_9H_{15}) could be rationalized by cleavage e and indicated the absence of a hydroxyl group in ring A (5).

The chemical shifts in the ^{1}H- and ^{13}C-nmr spectra of **1** closely resembled those of $\Delta^{20(29)}$ lupene derivatives (3,6), particularly the chemical shifts and multiplicity of H-19β (δ 2.38, ddd, J = 10.6, 10.6, and 5.3 Hz) and the chemical shifts of various carbons of rings D and E.

The remaining problem was to locate the position of the hydroxyl group in ring B. Of the possible locations C-6 and C-7, the former could be eliminated on the basis of a D_2O exchange experiment performed on **2**. In its ^{1}H-nmr spectrum two protons were exchangeable with D_2O, confirming the presence of the carbonyl group in **2** (and hence the equatorial hydroxyl group in **1**) at C-7. Final authentication was made by the coupling interaction of the α axial carbinylic proton with neighboring axial and equatorial protons at C-6, thereby giving rise to a pair of doublets centered at δ 3.79 ($J_{ax,ax}$ = 9.8 Hz, $J_{ax,eq}$ = 5.1 Hz). Similar coupling values were reported earlier in the case of loranthol, a lup-20(29)-ene with a 7β-hydroxyl group (5).

The heteronuclear ^{1}H-^{13}C chemical shift correlation spectrum (hetero-COSY) correlated the chemical shifts of various carbon atoms with their respective protons. The coupling interactions were illustrated by ^{1}H-^{1}H correlated spectroscopy (COSY-45°), which showed the connectivity of H-7α to both protons at C-6, and that of H-19α with H-18α as well as H_2-21.

The stereochemistry of psoracinol [**1**], particularly the β configuration of the hydroxyl function, was ascertained by nOe difference measurements at certain points in the molecule and was found to be in accordance with structure **1**. Irradiation at δ 3.79 (H-7α) resulted in 11.91% nOe at δ 1.02 (H_3-27), 3.87% nOe at δ 1.20 (H-5α), and 5.12% nOe at δ 1.29 (H-9α). Irradiation at δ 1.02 (H_3-27) resulted in 10.21% nOe at δ 3.79 (H-7α), 11.01% nOe at δ 1.38 (H-18α), 9.86% nOe at δ 1.29 (H-9α), 7.98% nOe at δ 1.52 (H_2-12), and 9.07% nOe at 1.41 (H_2-16). Irradiation at δ 2.38 (H-19β) resulted in 5.67% nOe at δ 0.78 (H_3-28) and 2.94% nOe at δ 1.26 (H-21). Finally, ir-

radiation at δ 0.78 (H_3-28) resulted in 4.98% nOe at δ 2.38 (H-19β), 5.27% nOe at 1.56 (H-13β), and 5.06% nOe at δ 1.27 (H_2-15). These nOe interactions are summarized in Figure 2.

To the best of our knowledge this is the first instance of the natural occurrence of a lupane derivative carrying a hydroxyl function at C-7 rather than the more usual 3 position, and its isolation may therefore be of chemotaxonomic significance.

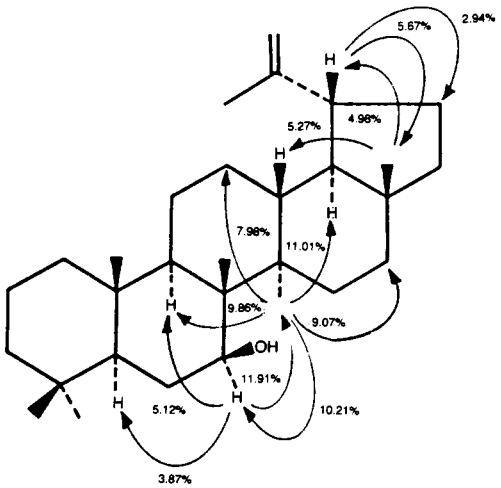


FIGURE 2. NOe interactions of psoracinol [**1**].

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's are uncorrected; ir spectra were recorded in $CHCl_3$ with a Jasco IRA-1 spectrophotometer and hrms on a Finnigan MAT 312 double focusing mass spectrometer coupled to a PDP 11/34 computer system. ^{1}H-nmr spectra were recorded on a Bruker Aspect AM-300 spectrometer with TMS as internal reference. The DEPT experiments were carried out with $\theta = 45°$, 90°, and 135°; the quaternary carbons were determined by subtraction of these spectra from the broad band ^{13}C-nmr spectrum.

For nOe measurements, the sample was frozen under liquid N_2 and degassed. A lower decoupler power of maximum 0.2 W with 35 attenuations in decibels was used. The pre-irradiation time was 11 sec, which is the sum of three delays as used in the nOe difference program of Bruker. The impulse length of 10 microsec was maintained to avoid saturation. The 2D COSY-45° experiments were acquired at 300 MHz with a sweep width of 4000 Hz (2K data points) in ω_2 and 2000 Hz (256 t_1 values zero-filled to 1K) in ω_1. The heteronuclear 2D ^{1}H-^{13}C chemical shift correlation experiments were carried out at 300 MHz with a sweep width of 12,820 Hz (2K data points) in ω_2 and 1024 Hz (256 t_1 values zero-filled to 2K) in ω_1. In both 2D experiments a 2-sec relaxation delay was used, and 16 transients were performed for each t_1 value.

PLANT MATERIAL.—The plant material was collected from the Karachi region and identified by Prof. S.I. Ali, the plant taxonomist, Department of Botany, University of Karachi. A voucher specimen has been deposited in the Herbarium of the Department of Botany, University of Karachi.

ISOLATION PROCEDURES.—The freshly collected plant material (20 kg) was extracted with EtOH at room temperature. The gummy residue obtained from the EtOH extract was partitioned between $CHCl_3$ and H_2O. The hexane-soluble portion of the $CHCl_3$ fraction was saponified with 5% alcoholic potash and worked up by the usual procedure. The nonsaponifiable fraction was subjected to cc over Si gel, eluting with a solvent gradient of increasing polarity. The last ten fractions eluted with hexane-$CHCl_3$ (6:4) were combined. The residue obtained on removal of solvent was dissolved in C_6H_6 and kept at 4°. The resulting colorless crystals were filtered off and resolved into two components through preparative tlc on Si gel using hexane-$CHCl_3$ (1:1) as the developing solvent. These were identified as angelicin and psoralen by direct comparison of their spectral data with those already reported in the literature (2,7,8).

The mother liquor left over after removal of the above crystals was rechromatographed over activated Si gel. The eluate obtained with hexane-$CHCl_3$ (92:8) was further purified through fractional crystallization from MeOH/C_6H_6 to afford compound **1** (yield 55.4 mg): ms *m/z* (rel. int.) $[M]^+$ 426 (28),

$[M-Me]^+$ 411 (13), $[M-H_2O]^+$ 408 (7), $[M-Me-H_2O]^+$ 393 (4), $[M-43]^+$ 383 (5), [ion d]$^+$ 220 (11), [ion c]$^+$ 217 (47), [ion b]$^+$ 207 (38), [ion a]$^+$ 189 (54), [ion d $-H_2O$]$^+$ 202 (37), [ion e]$^+$ 123 (75); ^{1}H-nmr ($CDCl_3$) δ 4.57 (1H, br s, H-29a), 4.68 (1H, br s, H-29b), 3.79 (1H, dd, $J_{eq,ax}=5.1$ Hz, $J_{ax,ax}=9.8$ Hz, H-7α), 1.67 (3H, s, H-30), 1.06 (3H, s, H-26), 1.02 (3H, s, H-27), 0.96 (3H, s, H-25), 0.78 (3H, s, H-28), 0.84 (3H, s, H-23), 0.81 (3H, s, H-24); ^{13}C-nmr ($CDCl_3$) C-1 (40.40), C-2 (18.37), C-3 (42.21), C-4 (33.71), C-5 (56.17), C-6 (37.13), C-7 (77.30), C-8 (48.60), C-9 (50.50), C-10 (37.24), C-11 (20.97), C-12 (25.27), C-13 (38.12), C-14 (42.89), C-15 (27.40), C-16 (35.51), C-17 (43.00), C-18 (48.36), C-19 (47.92), C-20 (151.05), C-21 (29.81), C-22 (40.09), C-23 (27.99), C-24 (15.95), C-25 (16.12), C-26 (12.89), C-27 (16.17), C-28 (18.02), C-29 (109.37), C-30 (19.30). The ^{13}C assignments follow from a number of $\Delta^{20(29)}$ lupene derivatives as model compounds (6).

OXIDATION OF PSORACINOL.—Psoracinol (20 mg) was dissolved in Me_2CO (40 ml) and treated with freshly prepared Jones reagent (50 ml). The reaction mixture was stirred at room temperature until the reaction was complete (tlc monitoring). It was diluted with H_2O and extracted with Et_2O. Removal of solvent from the Et_2O extract and crystallization from EtOH yielded psoracinone [**2**] (17.68 mg): mp 181°; [α]D +37° ($c=0.11$, $CHCl_3$); ir 1710, 3075, 1635, 880 cm^{-1}; hrms 424.3714 ($C_{30}H_{48}O$); ms *m/z* (rel. int.) $[M]^+$ 424 (15), $[M-Me]^+$ 409 (20), $[M-43]^+$ 381 (10), [ion c]$^+$ 217 (53), [ion d]$^+$ 218 (35), [ion b]$^+$ 205 (75), [ion a]$^+$ 189 (40), [ion e]$^+$ 123 (81); ^{1}H-nmr ($CDCl_3$) 4.56 (1H, br s, H-29a), 4.68 (1H, br s, H-29b), 1.65 (3H, s, H-30), 1.29 (3H, s, H-26), 1.13 (3H, s, H-27), 1.07 (6H, s, H-25 and H-23), 1.02 (3H, s, H-24), 0.81 (3H, s, H-28).

REDUCTION OF PSORACINONE.—To a solution of psoracinone (15 mg) in MeOH, $NaBH_4$ (4.0 mg) was added, and the mixture was refluxed for 24 h. MeOH was removed, and the resulting residue was treated with H_2O and extracted with $CHCl_3$. Drying and removal of the solvent from the organic phase gave the parent alcohol **1** which was repeatedly crystallized from C_6H_6/MeOH, mp 192°; [α]D +19.2° ($c=0.289$, $CHCl_3$).

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Received 16 January 1989