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Phytochemical Investigation of *Cocculus pendulus* stem

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Two new sterols, coccupendulusterols A and B, and an unknown aliphatic hydrocarbon have been isolated along with two reported alkaloids, pendulinin and cucsulinine and hexacosane from the stem of *Cocculus pendulus*. The structures of the new phytoconstituents have been established respectively as 7,8-*seco*-stigmast-11, 20(22)-diene-3 β-ol. stigmast-5,20(22)-diene-9 α-ol and 25-methyl tritriacont-21-ene-11-one-1-ol on the basis of spectral data analyses and chemical means.

COCCULUS pendulus (Forsk) Diels, Syn. *C. leaeba* DC (Minispermaceae), a scandent, slender, puberulous shurb growing in the dry parts of North and Western India[1,2]. which showed anticancer[3], hypertensive[3], tonic[4] and antiperiodic[4] properties, is generously rich in bisbenzylisoquinoline alkaloids[5-10]. The present communication deals with an extensive investigation of basic and neutral components of the stem of the plant.

EXPERIMENTAL

Extraction and isolation : The air-dried and powdered stem (2.5 kg), collected from the Jahanpanah City Forest, New Delhi[2], was extracted in a Soxhelt with ethanol (95%). The reddish brown viscous mass (105 g) was dissolved in 10% HCl. The acidic solution was filtered, extracted with $CHCl_3$ (3 x 100 ml) and basified with NaOH. The aqueous solution was re-extracted with $CHCl_3$ (3 x 150 ml), the organic phase dried over Na_2SO_4 and concentrated yielding a brown mass (20 g). This material was chromatographed over basic Al_2O_3 column after formation of a slurry and eluted with $CHCl_3$ containing increasing polarity of MeOH to isolate two known alkaloids, pendulinin ($CHCl_3$ - MeOH eluents, 95:5), 40 mg and cocsulinine ($CHCl_3$-MeOH, 9:1, eluants), 45 mg, identified by comparing M.P. and spectral data.

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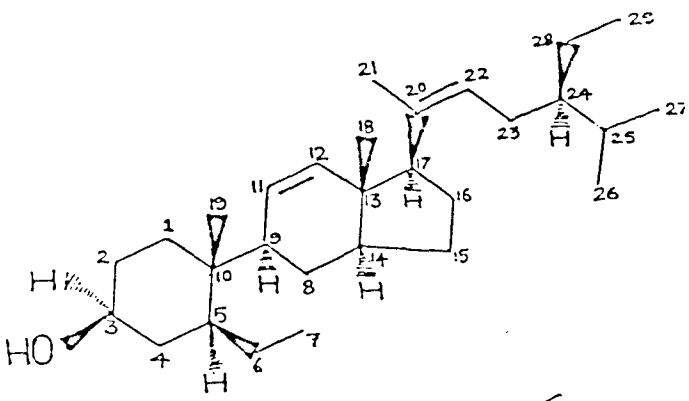
The acid insoluble material (65 g) was subjected to Si gel column which was eluted with petroleum ether, $CHCl_3$ and MeOH in order of increasing polarity to isolate the following compounds:

Hexacosane : Elution of the column with petroleum ether gave hexacosane, 15 mg, M.P. and M.M.P. 56-57°, co-TLC comparable.

Cocupendulusterol A (1) : Elution of the column with $CHCl_3$ afforded white flakes of 1, recrystallized from $CHCl_3$ - MeOH (1 : 1), 80 mg, M.P. 122 - 124°, UV λmax 205 nm (log ε 5.6), IR ϑ max (KBr) 3455 (OH), 2945, 2850, 1615 (C=C), 1435, 1365, 1190, 1045, 955, 800 cm^{-1}. ^1H NMR (100 MHz, $CDCl_3$) δ 5.37 (1 H, br, s, H-12),5.32 (1 H, br s, H-11), 5.09 (1 H, t, J = 6.10 Hz, H-22), 3.44 (1 H, br m, $W^1/_2$ = 14.0Hz, H-3∝), 2.35 (1 H, br s, H-9, 2.08 (2 H, br, s, H_2 - 23), 1.54 (3 H, br s, Me-21), 1.01 (3 H, br s, Me-19), 0.87 (3 H, br s, Me-29), 0.84 (3 H, d, J=6.0 Hz, Me-27), 0.79 (3 H, d, J = 6.0 Hz, Me-26), 0.70 (3 H, br s, Me-7), 0.68 (3 H, br s, Me-18). EIMS m/z (rel. int.) 414 $[M]^+$ ($C_{29}H_{50}O$) (69.5), 399 (15.4), 394 (53.6), 381 (83.3), 340 (2.3), 333 (32.9), 328 (11.9), 305 (2.1), 304 (16.1), 289 (23.3), 283 (15.2), 273 (33.1), 260 (5.0), 157 (18.1), 255 (59.4), 246 (3.0), 242 (7.2), 220 (8.9), 213 (54.6), 209 (2.0), 205 (13.0), 199 (9.1), 195 (2.4), 189 (23.1), 186 (10.8), 168 (11.6), 154 (2.9), 141 (2.0), 139 (4.6), 123 (6.9), 94 (71.3), 86 (8.4), 81 (22.6), 72 (23.1). Monoacetyl product with Ac_2O-Pyridine, M.P., 109-111° IR ϑmax (KBr) 1725 cm^{-1}. 3-Oxo product with Jones reagents, IR ϑmax 1705 cm^{-1}.



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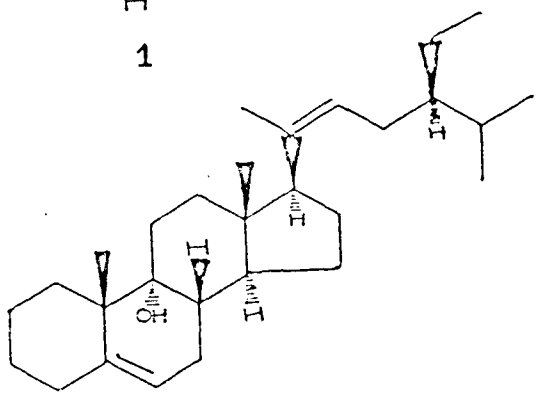

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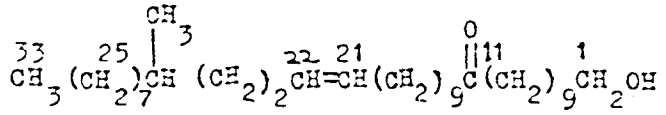

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Coccupendulusterol B (2) : Fractions eluted with $CHCl_3$ - MeOH (19:1) furnished colourless flakes of 2, 140 mg, M.P. 136 - 138°, UV λmax (MeOH) 205 nm (log ε 6.7), IR ϑ max (KBr) 3410 (OH), 2950, 1645 (C=C) 1450, 1375, 1320, 1255, 1055, 965, 805 cm^{-1}. ^1H NMR (100 MHz, $CDCl_3$) δ 5.37 (1 H, m, H-6), 5.08 (1 H, M, H-22), 3.48 (1 H, D_2O exchangeable, OH), 2.08 (2 H, br s, H_2-23), 1.52 (3 H, br s, Me-21), 1.01 (3 H, br s, Me-19), 0.88 (3H, br s, Me-29), 0.84 (3H, d, J = 6.0 Hz, Me-27), 0.79(3H, d, J = 6.0 Hz, Me-26), 0.68 (3H, br s, Me-18), EIMS m/z (rel. int.) 412 $[M]^+$ ($C_{29}H_{48}O$) (59.8), 397 (73.0), 394 (57.9), 382 (24.8) 359 (1.7), 357 (2.0), 345 (3.1), 330 (21.1), 304 (8.3), 301 (11.3), 290 (16.7), 273 (25.0), 258 (4.5), 255 (76.3), 248 (3.7). 240 (3.8), 230 (25.1), 228 (11.9), 214 (15.4), 212 (5.8), 200 (20.1) 198 (12.8), 190 (7.6), 184 (8.5), 176 (18.1), 164 (8.0), 148 (24.1), 134 (23.1),134 (23.2), 122 (19.7), 121 (46.2), 108 (58.6), 105 (78.5), 67 (20.1), 55 (100).

Aliphatic hydrocarbon (3) : Further elution of the column with $CHCl_3$ - MeOH (9:1) gave colurless crystals of 3,30 mg, M.P. 84-86°, IR ϑmax (KBr) 3455, 2915, 2855, 1700 (C=0), 1610 (C=C), 1460, 1205, 1145, 1070, 975, 915, 765, 735 cm^{-1}. ^1H NMR (100 MHz, $CDCl_3$) 5.35 (2 H, m, 2x CH=C), 4.05 (1 H, d, J - 10.5 Hz, CH_2OH-a), 3.82 (1H, d, J = 10.5 Hz, CH_2OH-b), 2.34 (2H, br s, CH_2CO), 2.27 (2 H, br s, CH_2CO), 2.03 (10 H, m, 5 x CH_2), 1.85 (1 H, br s, HC-25), 163 (2 H, br s, CH_2), 1.25 (38 H, br s, 19 x CH_2), 0.93 (3 H, d, J= 6.0 Hz, Me), 0.82 (3 H, t, J = 6.0 Hz, Me). EIMS m/z 506 $[M]^+$ ($C_{32}H_{66}O_2$) (3.6), 365 (2.1), 311 (2.2), 195 (4.6), 185 (11.3), 169 (9.8), 157 (12.2), 141 (18.2), 113 (35.3), 99 (55.3), 85 (60.3), 55 (100). Monoacetyl product, M.P. 70-71°, IR ϑmax 1724, 1700 cm^{-1}.

RESULTS AND DISCUSSION

Compound 1, named coccupendulusterol A, gave positive Lieberman - Burchard (L.B.) test for sterols, showed characteristic IR absorptions for hydroxyl group and unsaturation. The ^1H NMR spectrum of 1 displayed three down field singals for H-11, H-12 and H-22 olefinic protons, a 3∝-carbinol signal, a C-21 methyl singal attached to the olefinic carbon, two signals for C-7 and C-29 primary methyls, two signals for C-26 and C-27 secondary methyls and two signals for C-18 and C-19 tertiary methyls. The mass sepctrum of 1 showed a molecular ion peak corresponding to the steroidal formula $C_{29}H_{50}o$ indicating five degrees of unsaturation; two of them were adjusted in the olefinic linkages and three in the seco-ring steroidal skeleton. The spectrum showed diagnostically important peaks for $[M - Me]^+$, $[M - H_2O)]^+$, $[M - H_2O - Me]^+$, $[M - C_{10}H_{19}$, side chain, $SC]^+$, $[275 - Me]^+$, $[275 - H_2O]^+$, $[257$ - ring $D]^+$ $[213 - Me]^+$ supporting the unsaturated nature of the side chain containing an ethyl group at C-24 on biogenetic analogy and the hydroxyl group in the steroidal carbon framework. The ion fragments at m/z 273 and 141, generated due to cleavage of C_9 - C_{10} bond, suggested C_7 - C_8 *seco*-B-ring. The ion peaks at m/z 340, 72 328, and 86, arose due to cleavage of ring A, reflected the presence of the hydroxyl group in ring A which was placed at C-3 on the basis of biogenetic considerations. The ion fragments appearing at m/z 154, 260, 168, 246, 194, 220, 195, 333, 305 and 209, formed due to fission of rings C and D, attested the existence of $\Delta^{11(12)}$ olefinic linkage as observed in pluchiol

isolated from *Pluchea lanceolata*[11]. Acetylation of 1 yielded a monoacetyl product with acetic anhydride and pyridine. Treatment of 1 with Jones reagents yielded 3-oxo derivative which responded positively to Zimmermann test[12] for 3-oxo compound. Based on these evidences, the structure of the new sterol 1 has been established as 7,8-*seco*-stigmast-11, 20(22)-diene-3 β-ol.

Compound 2, designated coccupendulusterol B, positive to L.B. test, M^+ for $C_{29}H_{48}O$, had six olefinic linkage equivalents. Its IR spectrum demonstrated the presence of hydroxyl group and unsaturation. The ^1H NMR spectrum of 2 displayed signals for two H-6 and H-12 olefinic protons, a C-21 methyl signal attached to the olefinic carbon and five other methyl signals. Absence of any signal in the range δ 3.00 - 4.50, except a D_2O-exchangeable signal, suggested tertiary nature of the hydroxyl group. The mass spectrum of 2 showed important ion peaks for [M - Me]$^+$, [M - H_2O]$^+$, [397 - Me]$^+$, [M -$C_{10}H_{19}$, SC]$^+$, [273 - Me]$^+$, [273 - H_2O]$^+$ and [255 - ring D]$^+$ supporting unsaturated nature of the side chain with C-24 ethyl group. The ion fragments at m/z 357, 359, 55, 304, 108 and 345, formed due to cleavage of rings A and B, ruled out the presence of the hydroxyl group in rings A at C-3 and supported the C-5 olefinic linkage. The existence of the hydroxyl group at C-9 was established by generation of ion tragments at m/z 290, 122, 248, 164,148, 176, 134, 190, 228, 184, 214, 198, 200 and 212 due to cleavage of rings C and D. The compound resisted acetylation with acetic anhydride and pyridine and oxidation with Jones reagent attesting tertiary nature of the hydroxyl group. These data led to establish the structure of this new sterol as stigmast-5, 20(22)-diene-9 ∝-ol.

The aliphatic hydrocarbon 3, M^+ at m/z 506 ($C_{32}H_{66}O_2$), showed IR absorptions for hydroxyl and carbonyl groups, olefinic linkage and long aliphatic hydrocarbon. Its ^1H NMR spectrum displayed signals for olefinic protons, hydroxylmethylene and other methylene and methyl groups. A large numbr of fragment ions recorded in the mass spectrum exhibited a uniform differences of 14 mass units and decreasing in intensity with increasing molecular weight, thus confirming the presence of a long chain aliphatic compound. The prominent ion peaks for the fragments $[C_8H_{17}]^+$, $[C_9H_{19}]^+$ and [M - 141]$^+$ suggested the presence of a branched methyl group at C-25. The olefinic linkage at C-21 was deduced from the intensified ion peaks at m/z 169 [C_{22} - C_{23} fission]$^+$ and 195, 311 [C_{20} - C_{21} fission]$^+$. The major ion peaks for the fragments [$C_{10}H_{20}$ - OH]$^+$ and [CO-$C_{10}H_{20}$-OH]$^+$ indicated the location of the carbonyl group at C-11. The compound 3 formed a monoacetyl derivative with acetic anhydride - pyridine thus supporting the presence of an acetylable hydroxyl group in the molecule. The data led to formulate the structure of this natural product as 25-methyl tritriacont-21-ene-11-one-1-ol. These three natural products are being reported for the first time from a natural or synthetic source.

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REFERENCES

1. Kirtikar, K. R. and Basu, B. D., In; Basu, L. M. Ed, **Indian Medicinal Plants**, 2nd Ed, Vol. I, Periodical Experts Book Agency, Allahbad, 1933, 88.
2. Sharma, M.P. and Ahmed, J., **Indian J. Forestry**, 1991, 14, 76
3. Bhakuni, D.S., Dhar, M.L., Dhar, M. M., Dhawan, B. N. and Mehrotra, B.N., **Indian J. Exptl. Biol.**, 1969, 1, 250.
4. Nadkarni, K. M. In; **Indian Materia Medica**, 3rd Ed., Vol I, Popular Book Depot Bombay, 1954, 360.
5. Joshi, P. P., Bhakuni, D. S. and Dhar, M. M., **Indian J. Chem.**, 1974, 12, 517.
6. Bhakuni, D.S. and Joshi, P.P., **Tetrahedron**, 1975, 31, 2575.
7. Ikram, M., Shafi, N. and Abu Zarga, M. H, **Planta Medica**, 1982, 45, 253.
8. Husain, S.F., Khan, L., Guinaudeau, H., Leet, J. E., Freyer, A. J. and Shamma, M., **Tetrahedron**, 1984, 40, 2513.
9. Schiff, P. L. Jr., **J. Nat. Prod.**, 1983, 46, 1.
10. Jain, S., Shukla, K. and Bhakuni, D. S., **Fitoterapia**, 1991, 62, 391.
11. Alam, M. S., Chopra, N., Ali, M., Niwa, M. and Sakae, T., **Phytochemistry**, 1994, 37, 521.
12. Barton, D.H.R. and Mayo, P. de, **J. Chem. Soc.**, 1954, 1, 887.