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LANOSTANE TRITERPENES OF *CANSCORA DECUSSATA*

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Key Word Index—*Canscora decussata*; Gentianaceae; gluanone; canscoradione; *n*-alkanes; *n*-alkanols; friedelin; friedelan-3β-ol; β-amyrin; sitosterol; stigmasterol; campesterol.

Abstract—From the aerial parts of *Canscora decussata* Schult (Gentianaceae), five triterpenes, viz. gluanone, canscoradione, friedelin, fridelan-3-β-ol, and β-amyrin, three sterols, viz. sitosterol, stigmasterol, and campesterol, besides liberal amount of a mixtures of *n*-alkanes (C_{27}–C_{31}) and *n*-alkanols (C_{26}–C_{32}) have been isolated. The identity of the compounds has been established by chemical transformations, spectral evidence, and by direct comparison, where possible, with authentic reference materials. Gluanone and canscoradione have not been encountered before in nature.

INTRODUCTION

THE ISOLATION of nearly two dozen polyoxygenated (tri-, tetra-, penta- and hexa-) xanthones from the roots of *Canscora decussata* Schult (Gentianaceae) has been previously reported.[1–5] This paper describes the isolation of the triterpenes and sterols, and the ubiquitous *n*-alkanes and *n*-alkanols from the aerial parts of *C. decussata*, and characterization of the compounds by chemical and spectral methods.

RESULTS AND DISCUSSION

From the petrol. extract of the stems, leaves, and flowers of *C. decussata* the neutral fraction was separated from the weakly acidic methoxy xanthones[4] in the usual way. The neutral fraction afforded four different types of compounds, e.g. *n*-alkanes, *n*-alkanols, triterpenes, and sterols, by repeated column and preparative layer chromatography. Among these compounds, two triterpenes were found to be new naturally occurring substances; their structure elucidation is described below in order of their isolation.

Petroleum eluates of the second column chromatographic runs gave a colourless solid, consisting of a mixture of *n*-alkanes (minor constituent) and two triterpenes, R_f 0·38 and 0·45 (benzene). Repeated crystallization of the mixture from MeOH, in which the *n*-alkanes remained insoluble, followed by preparative layer chromatography, yielded the major triterpene, R_f 0·45, as a single entity, $C_{30}H_{48}O$ (M^+, 424), m.p. 178–80°, $[\alpha]_D^{30}$ +4·5° ($CHCl_3$). It responded to the Liebermann–Burchardt test for triterpenes and gave a positive tetranitromethane test. The IR spectrum showed characteristic bands at ν_{max} 1715 (6-ring

* Part VII in the series "Chemical Constituents of the Gentianaceae". For part VI see Ref. 5.

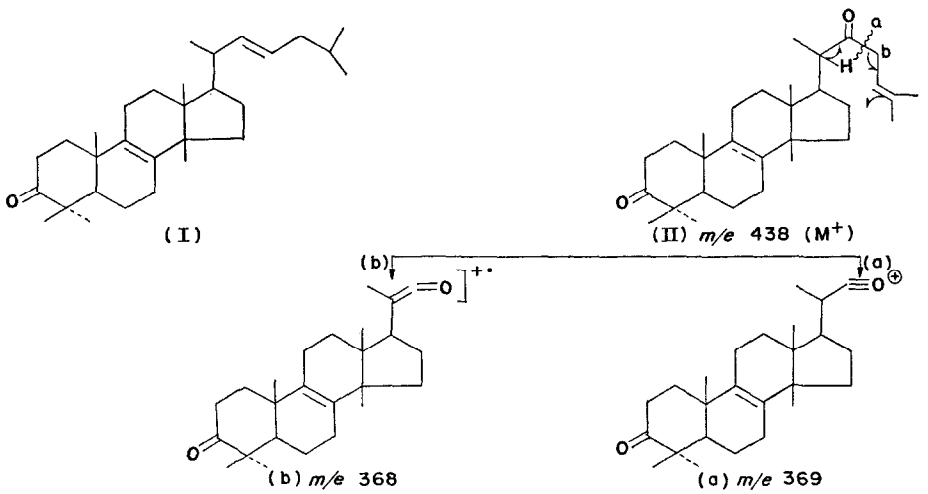
[1] CHAUDHURI, R. K. and GHOSAL, S. (1971) *Phytochemistry* **10**, 2425.
[2] GHOSAL, S., CHAUDHURI, R. K. and NATH, A. (1971) *J. Indian Chem. Soc.* **48**, 589.
[3] GHOSAL, S., CHAUDHURI, R. K. and BHATTACHARYA, S. K. (1972) *Abstracts, 8th IUPAC: Chemistry of Natural Products*, p. 78, NISI, New Delhi.
[4] GHOSAL, S., CHAUDHURI, R. K. and NATH, A. (1973) *J. Pharm. Sci.* **62**, 137.
[5] GHOSAL, S. and CHAUDHURI, R. K. (1973) *Phytochemistry* **12**, in press.

ketone), 1376 and 1364 (gem-dimethyl), and 970 cm^{-1} (Δ^{22}-*trans* unsaturation).[6] It had no UV maximum in the region λ210–260 nm indicating the absence of a conjugated ketone system. The PMR spectrum of the compound showed 48 protons, 24 of which were associated with eight methyl groups (0·70–1·1 ppm). This observation together with the existence of a side chain at C_{17} (from mass fragmentation data), and the oxygen as the ketonic function in a C_{30} molecule indicates the compound to be a tetracyclic triterpene ketone (I). The two methyls at C_{10} and C_{13} positions appeared, as expected,[7] at 0·90 and 0·70 ppm, respectively. A two-proton signal, centered at 5·2 ppm had the appearance of an unresolved triplet and is ascribed to the vinyl protons at C_{22}–C_{23}. The complex signals due to two protons around 2·4 ppm were similar in shape to those due to the methylene protons α- to the 3-keto group of a number of tetracyclic triterpenes.[8] The MS of I showed, aside from the molecular-ion peak at 424 (60%), significant fragment-ion peaks at *m/e* 409 (M-15, 22%, *m** 394·5), 381 (M-43, 100%, *m** 342·5), and 313 (M-111, 14%), presumably, due to the loss of Me, C_3H_7, and C_8H_{15} fragments from the side chain at C_{17}. The fragmentations are reminiscent of those reported[9,10] for a number of tetracyclic triterpenes having a side chain at C_{17}. The other significant fragment ion peaks at *m/e* 396 (4%), 382 (31%), 273 (11%), 271 (5%), 245 (42%), and 205 (44%) are ascribable to the loss of fragments CO, CH_2O, $C_{11}H_{19}$, $C_{11}H_{21}$, $C_{13}H_{23}$, and $C_{16}H_{27}$, respectively, from the molecular-ion. The position of the second double bond in I is located at $\Delta^{8(9)}$ on the basis of the PMR spectral evidence and the abundant *m/e* 245 peak in its MS. This fragment-ion peak was shifted, as expected, to *m/e* 247 in the corresponding alcohol. I, upon reduction with sodium borohydride in methanol, afforded the corresponding alcohol, $C_{30}H_{50}O$ (M^+, 426), m.p. 173°. It showed characteristic IR bands at ν_{max} 3395 and 1035 cm^{-1} for the OH group. The MS fragments of the alcohol exhibited, as expected, a difference of 2 m.u. from those of the ketone in all the significant peaks associated with the A-ring, *m/e* 275, 247, 207. On the basis of the above data, the compound has the structure (I), identical to that of gluanone which has been recently obtained as a transformation product of a new naturally occurring triterpene, 3-β-acetoxy-13α,14β,17β(H), 20α(H)-lanosta-8,22-diene.[11,12]

The other new triterpene of *C. decussata*, forming the lower layer (R_f 0·38) in the preparative layer chromatography of (I) was obtained as a minor constituent, $C_{30}H_{46}O_2$ (M^+, 438), m.p. 164–165°. It showed a twin peak centered around ν_{max} 1715 cm^{-1} due to two carbonyl functions present in the molecule. The UV absorption maximum of the triterpene did not show any maximum in the region λ210–260 nm, indicating that the two carbonyl groups in this compound are not in conjugation with the two double bonds. The MS of the triterpene showed significant peaks at *m/e* 369 (100%) and 368 (27%) associated with the loss of fragments C_5H_9 and C_5H_{10} from the molecular-ion and forming the ion-fragments (a) and (b), respectively. The ion-fragments (a) and (b) presumably originate by way of mechanism shown (II → a or b). The mechanism has precedent in the cleavage of the C_{17}-side chain of several tetracyclic triterpenes and steroids after the electron impact.[9] The other significant fragment-ion peaks appeared at *m/e* 423 (M–Me), 410 (M–CO), 397 (M–C_3H_5), 382 (M–CO–CO), 313 (M–$C_8H_{13}O$) and at *m/e* 245 and 205 due to the second double bond

[6] EGLINTON, G. (1964) in *Physical Methods in Organic Chemistry* (SCHWARZ, J. C. P., ed.), p. 73, Oliver & Boyd, Edinburg.
[7] LAVIE, D., SHVO, Y. and GLOTTER, E. (1963) *Tetrahedron* **19,** 2255.
[8] CHEUNG, H. T. and WONG, W. C. (1972) *Phytochemistry* **11,** 1771; and references therein.
[9] AUDIER, H. E., BEUGELMANS, R. and DAS, B. C. (1966) *Tetrahedron Letters* 4341.
[10] MCLAFFERTY, F. W. (1963) *Mass Spectrometry of Organic Ions*, p. 637, Academic Press, New York.
[11] SEN, A. B. and CHOWDHURY, A. R. (1971) *J. Indian Chem. Soc.* **48,** 1165.
[12] Direct comparison with gluanone could not be made due to its[11] non-availability.

at $\Delta^{8(9)}$. Structure (II) for the new minor triterpene of *C. decussata* which we name canscoradione should, however, be regarded as tentative in view of the limited data obtained. The yield of the compound being very poor, the total quantity of the triterpene isolated was exhausted in the studies conducted.



The other three triterpenes from the aerial parts were identified as friedelin, friedelan-3, β-ol, and β-amyrin. The sterol fraction consisted of a typical mixture[13] of sitosterol-stigmasterol, and campesterol, while the *n*-alkanes and *n*-alkanols were mixtures of C_{27}–C_{31} (C_{29}-predominating) and C_{26}–C_{32} compounds (C_{28}-predominating), respectively.

The roots of *C. decussata* afforded essentially the same neutral constituents but in poorer yields.

Triterpene constituents are rather rare in members of the Gentianaceae, and the amounts also are generally very low. A recent survey[14] has shown that the yield and number of triterpenes recorded in this family are much lower than those of the usual triterpene-bearing families. In this respect, *C. decussata* deserves special mention, since, unlike many other gentianaceous plants, it not only elaborates liberal amount of triterpenes but also exhibits catholicity in their structural patterns. The co-occurrence of friedelane, oleanane, and lanostane (or euphane) group of triterpenes is thus a notable feature of this species and when wider chemical surveys can be made, it will be interesting to see whether or not some *Canscora* species will be found to contain similar triterpenes. This is for the first time that occurrence of friedelane and lanostane (or euphane) group of triterpenes has been demonstrated in the Gentianaceae.

EXPERIMENTAL

General. All m.ps were determined in open capillaries and were uncorrected. UV spectra were determined in aldehyde-free 95% EtOH. IR specta were determined in KBr pellets. 60 MHz PMR spectra were determined on a Varian A 60-D Spectrometer in $CDCl_3$,$(Me)_4Si$ was used as the internal standard. MS were recorded on a AEI MS-9 double focussing spectrometer with an ionising potential of 70 eV. Separation by column chromatography was carried out with neutral alumina (Brockmann, activity grade *ca.* III). TLC experiments were carried out with silica-gel G (E. Merck) using benzene as the developer, Liebermann–Burchardt reagent and I_2 vapour were used for the staining purposes.

[13] CHAUDHURI, R. K. and GHOSAL, S. (1970) *Phytochemistry* **9**, 1895.
[14] CHAUDHURI, R. K. (1972) Ph.D. Thesis, Banaras Hindu University, 99.

Isolation of triterpenes and sterols from C. decussata. Dried and finely powdered stems, leaves, and flowers (2·3 kg) of the plant were extracted with petrol. (60–80°) in a Soxhlet for 30 hr. The petrol. extract was concentrated (*ca.* 250 ml) and then extracted with aq. citric acid (12%, 200 ml) for 12 hr. The aq. citrate solution was kept aside for further processing for weakly basic constituents. The petrol. extract was evaporated, the residue dissolved in Et_2O (300 ml), and extracted with aq. NaOH (6%, 4 × 25 ml). The aq. alkaline solution was processed, as described earlier,[4] for weakly polar xanthones. The Et_2O layer was processed, in the usual way, when a colourless solid was obtained. The solid (2·1 g) showed several spots on analytical TLC plates. A portion of the solid (0·68 g) was dissolved in C_6H_6 (15 ml) and chromatographed over alumina (3 × 46 cm). Petrol., C_6H_6, and $CHCl_3$ (51 each) were used as the eluents. The residue (0·38 g). from the petrol. eluates (fraction A) showed three major spots due to *n*-alkanes and two triterpenes, viz. gluanone and friedelin. In addition, a feeble spot due to the minor triterpene, canscoradione, was observed. The residue (0·13 g) from the C_6H_6 eluates (fraction B) showed three major spots due to *n*-alkanols, friedelan-3β-ol, and β-amyrin. The residue (68 mg) from the $CHCl_3$ eluates (fraction C) showed two major and one minor spots due to sitosterol, stigmasterol, and campesterol. The presence of these components was detected on analytical TLC plates with suitable markers. The individual components were separated from the respective fractions by repeated chromatography (column and preparative layer) and fractional crystallization from suitable solvents.

Fraction A. The residue was dissolved in benzene and chromatographed over alumina. Petrol. (4 l.) was used as the eluent. Fractions (40 ml) were collected. The first four fractions afforded a mixture of *n*-alkanes (0·2 g) as a white microcrystalline powder (m.p., m.m.p., IR, M^+, C_{27}–C_{31}, $C_{29}H_{60}$ being the major component). Fractions 18–27 gave a mixture of *n*-alkanes (traces), gluanone, and canscoradione. Fractions 42–46 afforded friedelin (72 mg, m.p., m.m.p., co-TLC, reduction to friedelan-3β-ol) plus gluanone and canscoradione (traces).

Gluanone (I). The residue from fractions 18–27 crystallized from MeOH, in which *n-alkanes* were insoluble, as colourless needles (36 mg), m.p. 168–172°. It showed two spots on TLC, R_f 0·38 and 0·45. These were separated by preparative TLC. The upper layer, R_f 0·45, was eluted out with $CHCl_3$ and the residue recrystallized from MeOH as needles, m.p. 178–180° (Found: C, 84·48; H, 11·84. $C_{30}H_{48}O$ requires: C, 84·51; H, 11·78%).

$NaBH_4$ reduction of gluanone. Gluanone (20 mg) in MeOH (10 ml) was treated, portionwise, with $NaBH_4$ (150 mg). The reaction mixture was kept at ordinary temp. overnight. The solvent was evaporated and the residue was triturated with H_2O. The insoluble solid was filtered, dried, dissolved in C_6H_6, and chromatographed over alumina. Petrol.-C_6H_6 (1 : 1) eluates afforded a solid (16 mg) which crystallized from $CHCl_3$–MeOH as colourless needles, m.p. 173° (Found: C, 84·38; H, 12·12. $C_{30}H_{50}O$ requires: C, 84·51; H, 11·74%).

Canscoradione (II). The component, R_f 0·38, was obtained from the preparative TLC as a colourless solid (*ca.* 2 mg), m.p. 166–170°. The total neutral fraction, upon three successive preparative layer chromatography of the appropriate fraction, afforded a total of 7 mg of canscoradione. It crystallized from MeOH as needles, m.p. 164–165°.

Fraction B. The solid obtained from the fraction B was rechromatographed over alumina. Elution was carried out with petrol., C_6H_6, and different proportions of mixtures thereof. The first petrol.–C_6H_6 (1 : 1) eluates afforded friedelan-3β-ol which crystallized from MeOH as needles (21 mg, m.p., m.m.p., co-TLC, $[\alpha]_D$). The petrol.–C_6H_6 (1 : 4) eluates afforded the mixture of *n*-alkanols (C_{28}–C_{32}) which crystallized from acetone as needles (152 mg, m.p., m.m.p., IR, M^+, $C_{28}H_{58}O$ being the major component). The benzene eluates afforded β-amyrin which crystallized from acetone as needles, (42 mg, m.p., m.m.p., co-TLC, M^+, *m/e*, benzoate, hydrolysis of the benzoate).

Fraction C. Fractional crystallization of the residue from fraction C, from EtOH afforded stigmasterol as the first crop which was purified by repeated crystallizations from the same solvent. The final product was obtained as colourless needles (14 mg, m.p., M^+, *m/e*, acetate). EtOH mother liquor, upon concentration, gave sitosterol which crystallized from $CHCl_3$–MeOH as flakes (27 mg, m.p., M^+, *m/e*, acetate). From the mother liquors of the above two sterols, campesterol was obtained by preparative layer chromatography as colourless needles (8 mg, m.p., M^+, *m/e*, however, two small peaks at *m/e* 414 and 412, due to sitosterol and stigmasterol, respectively, were also observed).

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