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C-21 STEROIDS IN THE DEFENSIVE SECRETIONS OF SOME INDIAN WATER BEETLES—II*

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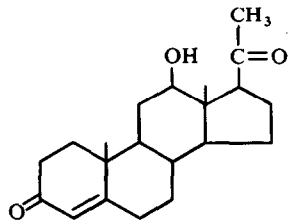
Abstract—From the defensive secretions of water beetles *Cybister limbatus*, *C. tripunctatus* and *C. confusus*, a number of C-21 steroids *viz.* Δ^4-pregnen-12β-ol-3,20-dione (I), $\Delta^{4,6}$-pregnadien-12β-ol-3,20-dione (II), Δ^4-pregnen-20β-ol-3-one (III), and Δ^4-pregnen-21-ol-3,20-dione (IV) have been isolated and characterized.

THE presence of some vertebrate hormones in the defensive secretions of water beetles (family Dytiscidae) has been reported by Schildknecht *et al.*[1] We have studied the secretions from the indigenous water beetles, *Cybister limbatus*, *C. tripunctatus* and *C. confusus* and have isolated a number of C-21 steroidal hormones from the same. From *C. limbatus*, in addition to the four C-21 steroids isolated earlier,[2] two 12β-hydroxy steroids, I and II, of rare occurrence have been isolated and characterized. From *C. tripunctatus*, III and IV were the major steroids whereas from *C. confusus*, IV was the only sizeable product obtained.

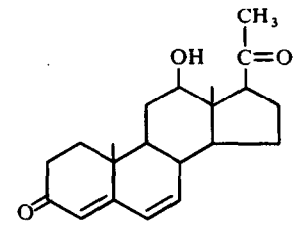
Cybister limbatus. The secretion collected from the prothoracic defensive glands of the beetles, on preparative TLC was separated into five major bands. From the slowest moving band, two compounds A and B were isolated.

Compound A shows IR absorptions for OH, α,β-unsaturated CO and an isolated but H-bonded CO group. The presence of an α,β-unsaturated CO group was confirmed by UV absorption at 241 nm. The molecular formula $C_{21}H_{30}O_3$ was deduced from its molecular ion peak at *m/e* 330. It was further recognised as a steroid with a C-21 skeleton. The fragmentation pattern was indicative of an Ac group at C-17 and a OH function. Since a NMR signal attributable to a single proton on O-bearing carbon (3·55 δ) was observed, the OH must be secondary. Assuming 17β-orientation for the Ac group, as is the case with naturally occurring steroids, and keeping in view that the CO of Ac is H-bonded, the only two possible positions for the OH group are 16β and 12β. 16β position was ruled out as the $C_{18}\underline{H}_3$ would have an NMR signal appreciably down field[3] compared to the observed value of 0·78 δ. The chemical shifts as calculated[4] for C_{18} and C_{19}-Me groups and their comparison with the observed values led us to assign the 12β orientation for the OH group. This was confirmed by acetylation using acetic anhydride/pyridine. Accordingly structure Δ^4-pregnen-12β-ol-3,20-dione (I) was assigned to compound A, which has hitherto not been isolated from either insect or plant source.

[2] A. T. Sipahimalani, V. R. Mamdapur, N. K. Joshi and M. S. Chadha, *Naturwissenschaften* **57**, 40 (1970). *schaften* **57**, 40 (1970) forms part I of this series.



I

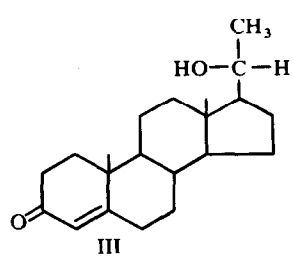


II

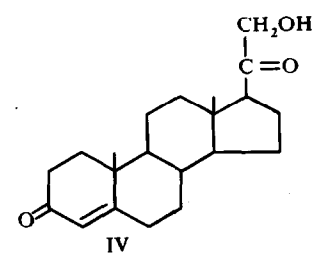
Compound B shows IR absorptions for OH, α,β,γ,δ-unsaturated CO and an isolated but H-bonded CO group. The UV absorption at 283 nm confirmed the presence of an α,β,γ,δ-unsaturated CO group. Its NMR spectrum shows all the groups present in I with an additional 2-proton signal at 6·20 δ. This was assigned to a double bond at C_6. The mass spectrum shows a molecular ion at *m/e* 328. The above data suggest that compound B as the dehydro of I is $\Delta^{4,6}$-pregnadien-12β-ol-3,20-dione (II). Schildknecht and Körnig[5] have reported the isolation and characterization of this steroid from a Mexican *Cybister* species and have kindly compared the IR spectrum of our sample with theirs and found the two identical.

Cybister tripunctatus. The secretion from the prothoracic region of the beetles was fractionated by preparative TLC. As a result two colourless solids (C, m.p. 169–171° and D, m.p. 138–141°), both giving positive steroidal colour tests were obtained.

Compound C shows absorptions for OH and an α,β-unsaturated CO group in the IR spectrum. The UV absorption at 241 nm (log ε, 4·2) supported the presence of an α,β-unsaturated CO group. The mass spectrum exhibited a molecular ion peak at *m/e* 316. The compound was recognised as a C-21 steroid having an OH group. A prominent ion peak at *m/e* 124, characteristic of Δ^4-3-keto steroids[6] was also observed. The NMR data of compound C agreed with that reported[7] for Δ^4-pregnen-20β-ol-3-one (III). Its identity as III was further confirmed through m.p., m.m.p. and IR comparison with an authentic sample.* The acetate of III was also found to be identical with authentic Δ^4-pregnen-20β-acetoxy-3-one. After the completion of our work Schildknecht *et al.*[8] reported the isolation of III from *Ilybius fenestratus*, as a minor component (1 μg/beetle) whereas it is the major compound of *Cybister tripunctatus* (100 μg/beetle).



III



IV

* An authentic sample was kindly supplied by Dr. J. Fried of Syntex Corporation, Palo Alto, Calif., U.S.A.

Compound D on the basis of spectroscopic data (IR, UV, NMR, MS) and its comparison with that of an authentic sample, was shown to be Δ^4-pregnen-21-ol-3,20-dione (Deoxycorticosterone; IV) first reported from an insect source by Schildknecht *et al.*[9]

Cybister confusus. The secretion from the prothoracic defensive glands of the beetles, fractionated by preparative TLC, yielded a single major component. It showed UV absorption at 241 nm and was characterized as Δ^4-pregnen-21-ol-3,20-dione (IV) by TLC and IR comparison with an authentic sample.

EXPERIMENTAL

M.ps are uncorrected. UV spectra were taken on a Zeiss RPQ 20A recording spectrophotometer. IR spectra were taken on a Perkin Elmer Infracord 137-B spectrophotometer. NMR spectra were determined with a Varian A-60A spectrometer using TMS as an internal indicator. Mass spectra were recorded on a CEC mass spectrometer, model 21-110B, using an ionising potential of 70 eV, an ionising current of 20 u amp, and the temp of 200°.

Water beetles were collected near Poona, Maharashtra, India. Milk-white secretions emanating from the prothoracic defensive glands were collected in glass capillaries. These were ground in MeOH and the extracts subjected to repeated TLC on 1 mm thick layers of a mixture of silica gel GF_{254} and H (1:1). The plates were prewashed in MeOH before they were activated at 100° for 1 hr.

Cybister limbatus (300 *numbers*). The extract on preliminary preparative TLC using cyclohexane–EtOAc (1:1) showed a number of bands (dark blue to blue in UV light). The major bands 1–5 having R_f values of 0·55, 0·45, 0·35, 0·30 and 0·15 were scraped and eluted with MeOH.

The slowest moving band (R_f 0·15) from its UV absorption at 241 and 283 nm was found to be a mixture. By multiple development chromatography (12 runs), using the hexane–acetone (3:1), it could be resolved into two compounds A (24 mg, 80 μg/beetle) and B (11 mg, 37 μg/beetle). These separated as semi-solid masses and it was therefore difficult to determine their m.ps.

Compound A: Δ^4-*pregnen*-12β-*ol*-3,20-*dione* (*I*). UV absorption: $\lambda_{max}^{CH_3OH}$ 241 nm; MS: M^+ ion *m/e* 330, other mass ion peaks at *m/e* 312, 297, 287, 269 and 254; IR (KBr): 3344, 1074, 1036 cm^{-1} (OH), 1670, 1613 cm^{-1} (α,β-unsaturated CO, strong 1670 cm^{-1} peak splits into 1690 and 1665 cm^{-1} when taken in $CHCl_3$); IR of acetate ($CHCl_3$): 1735, 1244 cm^{-1} (acetate), 1707 cm^{-1} (CO), 1665, 1620 cm^{-1} (α,β-unsaturated CO); NMR ($CDCl_3$): 0·78 δ ($C_{18}\underline{H}_3$), 1·20 δ ($C_{19}\underline{H}_3$), 2·22 δ ($C\underline{H}_3CO$), 3·55 δ (broad C<u>H</u>OH), 5·80 δ (vinylic proton at C_4). On the basis of this data Compound A was established as Δ^4-pregnen-12β-ol-3,20-dione (I).

Compound B: $\Delta^{4,6}$-*pregnadien*-12β-*ol*-3,20-*dione* (II). UV absorption: $\lambda_{max}^{CH_3OH}$ 283 nm; MS: M^+ ion *m/e* 328, other mass ion peaks at *m/e* 310, 295, 285, 267 and 252; IR (KBr): 3367, 1039, 1073 cm^{-1} (OH), 1676 cm^{-1} (H bonded C=O), 1660, 1617, 1585 cm^{-1} (α,β,γ,δ-unsaturated CO); NMR ($CDCl_3$): 0·83 δ ($C_{18}\underline{H}_3$), 1·14 δ ($C_{19}\underline{H}_3$), 2·25 δ ($C\underline{H}_3CO$), 3·60 δ (broad C<u>H</u>OH), 5·77 δ (vinylic proton at C_4); 6·20 δ (2 vinylic protons at C_6, C_7). Its IR spectrum was identical to that of II which was isolated by Schildknecht and Körnig[5] from Mexican *Cybister* species.

Cybister tripunctatus (400 *numbers*). Preparative TLC of the secretion from the prothoracic defensive glands was carried out. Using cyclohexane–EtOAc (1:1) (two repeated runs), two major bands having R_f values of 0·52 and 0·36 were observed in UV light. These were scraped and eluted with MeOH. The upper band (R_f 0·52) was further purified by preparative TLC using the above solvent system. This resulted in the isolation of colourless solid C, 39 mg (100 μg/beetle). Lower band (R_f 0·36) when purified in a similar manner gave colourless solid D, 57 mg (143 μg/beetle).

Compound C: Δ^4-*pregnen*-20β-*ol*-3-*one* (III). M.p. 169–171°; UV absorption: $\lambda_{max}^{CH_3OH}$ 241 nm (log ε, 4·2); MS: M^+ ion *m/e* 316, other mass ion peaks at *m/e* 298, 274, 124; IR (KBr): 3510, 1110 cm^{-1} (OH), 1667, 1612 cm^{-1} (α,β-unsaturated CO); NMR ($CDCl_3$): 0·81 δ ($C_{18}\underline{H}_3$), 1·20 δ ($C_{19}\underline{H}_3$), 1·15 δ ($C_{21}\underline{H}_3$, d, J = 6 c/s), 3·75 δ (broad C<u>H</u>OH), 5·80 δ (vinylic proton at C_4); NMR of its acetate ($CDCl_3$): 0·70 δ ($C_{18}\underline{H}_3$), 1·20 δ ($C_{19}\underline{H}_3$), 1·16 δ ($C_{21}\underline{H}_3$, d, J = 6 c/s), 4·86 δ (broad C<u>H</u>OAc), 5·80 δ (vinylic proton at C_4). A comparison (m.p., m.m.p. and IR) with an authentic sample showed it to be Δ^4-pregnen-20β-ol-3-one (III).

Compound D: Δ^4-*pregnen*-21-*ol*-3,20-*dione* (IV). M.p. 138–141°; UV absorption: $\lambda_{max}^{CH_3OH}$ 241 nm; IR (KBr): 3475 cm^{-1} (OH), 1693 cm^{-1} (H-bonded CO), 1668, 1613 cm^{-1} (α,β-unsaturated CO); NMR ($CDCl_3$) 0·70 δ ($C_{18}\underline{H}_3$), 1·20 δ ($C_{19}\underline{H}_3$), 4·20 δ (—CO—C<u>H</u>$_2$—O—), 5·80 δ (vinylic proton at C_4); MS of

acetate: M^+ ion *m/e* 372, other mass ion peaks at *m/e* 357, 330, 312, 299 and 271. It was identical (m.p., m.m.p., IR and NMR) with an authentic sample of Δ^4-pregnen-21-ol-3,20-dione (IV).

Cybister confusus (6 *numbers*). Preparative TLC of the secretion using cyclohexane–EtOAc (1:1) gave Δ^4-pregnen-21-ol-3,20-dione (IV) as a single major product.

Preparation of acetates of I, III *and* IV. 4–5 mg of each steroid was dissolved in 0·5 ml pyridine and 0·5 ml Ac_2O and allowed to stand at room temp for 48 hr. Excess reagents were removed by heating on a water bath under a stream of N_2. Dry residues were purified by preparative TLC over silica gel GF_{254}-H (1:1) using chloroform as an eluent.

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