Phytochemistry, Vol. 20, No. 6, pp. 1444–1445, 1981.
Printed in Great Britain.

0031-9422/81/061444-02 $02.00/0

AN *N*-GLYCOSIDE AND STEROIDS FROM *ARISTOLOCHIA INDICA**

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(*Received* 9 *October* 1980)

Key Word Index—*Aristolochia indica*; Aristolochiaceae; chemotaxonomy; phenanthrene derivative; aristololactam *N*-β-D-glucoside; steroid; 3β-hydroxy-stigmast-5-en-7-one; 6β-hydroxy-stigmast-4-en-3-one.

Abstract—A phenanthrene derivative, aristololactam *N*-β-D-glucoside, and the steroids 3β-hydroxy-stigmast-5-en-7-one and 6β-hydroxy-stigmast-4-en-3-one have been isolated from *Aristolochia indica*.

We have already reported the isolation and structure elucidation of some new phenanthrene derivatives [1] and a sesquiterpene [2] from the roots of *Aristolochia indica* which possesses interesting antifertility activity [3] besides being a known source of aristolochic acid (**1a**), a tumour-inhibitory principle. Further examination of the plant led us to the isolation of another new phenanthrene derivative and two rare, naturally occurring steroids.

The phenanthrene derivative was obtained from the alcoholic extract as a deep-brown, high-melting (> 320°), amorphous powder. Besides the molecular ion peak at *m/z* 441, the mass spectrum of the compound exhibited prominent peaks for the aglycone part at *m/z* 308, 292 and 279 which were 14 mu lower than those of the co-occurring aristololactam-β-D-glucoside (**2a**).

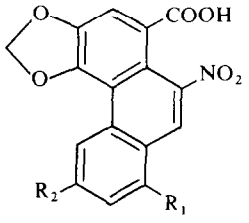
While direct acetylation (Ac_2O–pyridine) of the compound furnished a penta-acetate (M^+ at *m/z* 651), mp 146–148°, prior CH_2N_2 treatment provided a methyl ether tetra-acetate (Found: M^+ at *m/z* 623.1590; Calc. for $C_{31}H_{29}NO_{13}$: 623.1637), mp 254°, $[\alpha]_D$ −97.1° (*c* 0.7, $CHCl_3$), ν_{max}^{KBr} 1750, 1710, 1620, 1510, 1420, 1370, 1230, 1030 cm^{-1}, indicating the presence of a hydroxy group in the glycoside in lieu of the OMe group in **2a**. The IR spectrum of the methyl ether tetra-acetate showed the presence of an amide linkage (peak at 1710 cm^{-1}) and its ^1H NMR spectrum (80 MHz; FT; $CDCl_3$) could be best explained on the basis of structure **2b**. Thus, the two singlets at δ 6.28 (2 H) and 3.89 (3 H) could be assigned to a methylenedioxy and an aromatic methoxy function respectively and a doublet at the most down-field position (δ 8.00) to the C-5 proton, the coupling constant (J = 2 Hz) corroborating the presence of a substituent at C-6. The doublet for the C-8 proton was found at δ 7.72 (J = 9 Hz) while the one-proton singlet at δ 7.49 could be attributed to the C-2 hydrogen. The signals for the C-7 and C-9 hydrogens, which could not be located, must have merged with the strong solvent signal.

The compound was therefore concluded to be an *N*-glycoside (**2c**) of the aristololactam derived from aristolochic acid C (**1b**). Such glycosides are known [4] to be resistant to mineral acid hydrolysis. We could, however, effect the hydrolysis in one step by heating with 85% formic acid at 175° for 2 hr, rather than the two stage process of $LiAlH_4$ reduction followed by mild acid treatment. The sugar unit was then identified by paper chromatography as glucose. (The aglycone part was obtained in insufficient quantities for full characterization, but gave a correct molecular ion at *m/z* 293 in the mass spectrum.) Finally, the closeness of the specific rotation of the methyl ether tetra-acetate **2b** with that of **2a** tetra-acetate, and biogenetic considerations suggested it to be a β-D-glucoside, the complete structure of the compound thus being concluded to be **2c**.

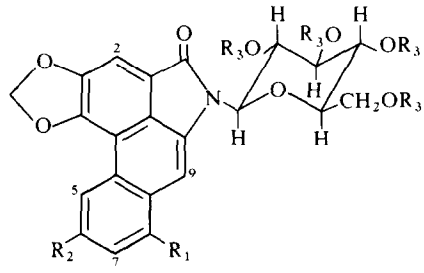
Petrol extraction of the roots yielded two isomeric minor steroidal constituents of molecular formula $C_{29}H_{48}O_2$ (associated with a small amount of the lower homologues), besides sitosterol and stigmast-4-en-3-one already reported [1]. Both contained a hydroxyl and a conjugated carbonyl function as evidenced from the IR, and saturated C_{10} side chains as revealed by their mass spectra.

From the fragmentation pattern, one of them (mp 132–134° (MeCN), ν_{max}^{nujol} 3530, 1670 cm^{-1}, λ_{max}^{EtOH} 236,302 nm) appeared to be 3β-hydroxy-stigmast-5-en-7-one (**3a**). It showed a very weak M − side chain − 42 peak (at *m/z* 245) and a series of peaks at *m/z* 205, 192, 187, 179 (weak), 168 and 161 expected [5] of such a system. The structure was confirmed by comparison of the compound and its acetate with authentic specimens. Although known synthetically for a long time, its first natural occurrence was reported only recently [6]. The other steroid (mp 208–210° (MeCN), ν_{max}^{nujol} 3500, 1690, 1620, 1040, 1020, 880 cm^{-1}) showed in its mass spectrum prominent M − side chain − 42 and M − side chain − 42 − H_2O peaks at *m/z* 245 and 227 respectively and a significant peak at *m/z* 152 characteristic [7] of a 3-keto-Δ^4-steroid hydroxylated at C-6. Indeed, Jones oxidation of the sample afforded (TLC) stigmast-4-ene-3,6-dione. A direct comparison of the natural steroid with an authentic specimen of 6β-hydroxy-stigmast-4-en-3-one (**3b**), recently isolated from a plant source [8], confirmed its identity.

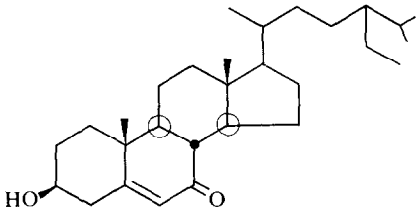
* Part 63 in the series "Studies on Indian Medicinal Plants". For Part 62 see Chakravarty, A. K., Pakrashi, S. C. and Uzawa, J. (1981) *Can J. Chem.* (in press).



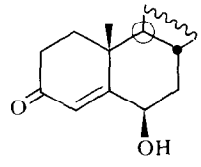
1a $R_1 = OMe$, $R_2 = H$
1b $R_1 = H$, $R_2 = OH$



2a $R_1 = OMe$, $R_2 = R_3 = H$
2b $R_1 = H$, $R_2 = OMe$, $R_3 = COMe$
2c $R_1 = R_3 = H$, $R_2 = OH$



3a



3b

Acknowledgements—We are grateful to Dr. F. C. Chang, University of South Alabama, U.S.A., for a comparison of samples and to Prof. A. Chatterjee, University of Calcutta, for a ^1H NMR spectrum.

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