Phytochemistry, 1969, Vol. 8, pp. 327 to 330. Pergamon Press. Printed in England

SHORT COMMUNICATION

# CONSTITUENTS OF *OLEANDRA WALLICHII*

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(*Received* 29 *May* 1968)

**Abstract**—A triterpene monohydroxy alcohol wallichiniol, its acetate and a dihydroxy triterpene alcohol have been isolated as minor constituents from the fern *Oleandra wallichii* and characterized. β-Sitosterol and sucrose have also been obtained from it.

RECENTLY we reported the isolation and characterization of some new triterpenes from the ferns, *Oleandra neriifolia*[1] and *O. wallichii*.[2] The results of further investigation with the minor constituents isolated from *O. wallichii* are recorded here.

Shade-dried and powdered whole plant (rhizomes, leaves and roots) was successively percolated with benzene and alcohol at room temperature. The concentrated benzene extract was partitioned between *n*-hexane and alcohol (90%) and the residue obtained in each case was chromatographed on alumina. Repeated chromatography and fractional crystallization yielded, besides the constituents reported earlier, a new triterpene alcohol wallichiniol, its acetate and a dihydroxy triterpene alcohol along with β-sitosterol. The alcohol extractive yielded sucrose.

*Wallichiniol*

$C_{30}H_{52}O$ (mol. wt., mass spectra, 428); m.p. 310–312°, $[\alpha]_D$ +12°; $\nu_{max}^{KBr}$ 3450 $cm^{-1}$ (OH). It gave positive LB and Noller's tests but no colour with tetranitromethane. With $Ac_2O$ and pyridine it formed a monoacetate $C_{32}H_{54}O_2$, m.p. 315–320°; $[\alpha]_D$ +43°; $\nu_{max}^{KBr}$ 1730, 1250 $cm^{-1}$. On oxidation with $CrO_3$ and AcOH it gave a ketone $C_{30}H_{50}O$, m.p. 285–287°, but no acid or aldehyde could be isolated from the reaction mixture. The formation of a monoacetate and oxidation to a ketone indicated the only hydroxyl group present in wallichiniol to be secondary. The ketone, when subjected to the Huang-Minlon modification of the Wolff-Kishner reduction,[3] yielded a saturated hydrocarbon $C_{30}H_{52}$, m.p. 208–713°. The molecular formula $C_{30}H_{52}O$ and the absence of a double bond in the molecule as well as the chemical evidence discussed above indicated the compound to be a saturated pentacyclic triterpene alcohol. Chemotaxonomic and biogenetic[4] considerations favoured a hopane or modified hopane type of carbon skeleton for the alcohol. However, a study in the cracking pattern of the mass spectra of wallichiniol revealed some interesting features.

[1] G. N. PANDEY and C. R. MITRA, *Tetrahedron Letters* **15**, 1353 (1967).
[2] G. N. PANDEY and C. R. MITRA, *Tetrahedron Letters* **47**, 4683 (1967).
[3] HUANG-MINLON, *J. Am. Chem. Soc.* **68**, 2487 (1946).
[4] G. BERTI, F. BOTTARI and A. MARSILLI, *Tetrahedron Letters* **1**, 1 (1964).

The mass spectrum of wallichiniol (Fig. 1) gave the molecular ion peak $M^+$ at *m/e* 428 and the base peak was observed at *m/e* 191. The successive peaks to $M^+$ were observed at *m/e* 413 and 395. Obviously, the peaks at *m/e* 413 and *m/e* 395 are due to the loss of a methyl (428-15) and a water molecule (413-18) thereby confirming the presence of a secondary hydroxyl group. A significant feature in the mass spectrum is that there is no indication of the loss of 43 mass units from the molecular ion $M^+$, equivalent to an isopropyl side-chain as in the case of hopane derivatives or lupanes. It has, however, been observed in case of lupenes that the loss of the vinyl group may not be always significant.[5] The conspicuous absence of any peak at *m/e* $M^+$-43 in the mass spectrum, therefore, rules out the possibility of a hopane or modified hopane type of carbon skeleton as well as those of lupane for wallichiniol.

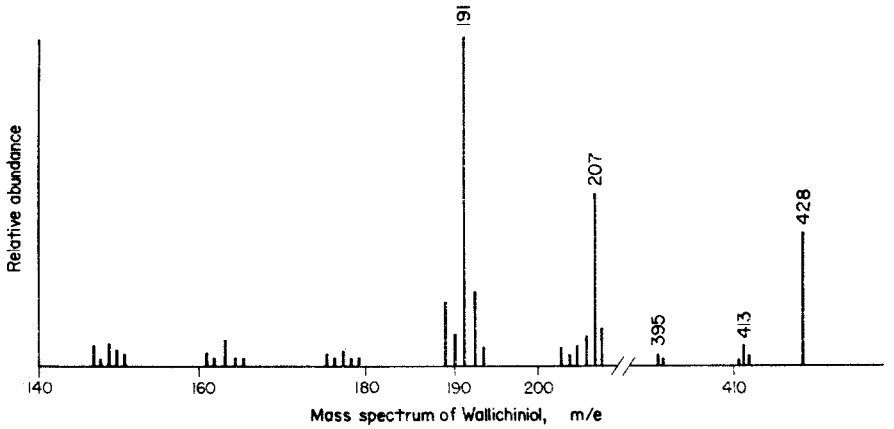


Fig. 1

The fragments appearing at *m/e* 207 and *m/e* 191 are due to those formed by the splitting of ring C and is analogous to the fragments formed in the mass spectra of saturated triterpenoids of oleananes, ursanes or lupanes[6] as also in neriifoliol,[1] the hydroxyl group being embedded on either side of the molecule, but they differ sharply from the fragments formed by those triterpenoids where methyl substituents are present at C-13 and C-14. It has been observed by Djerassi that when methyl substituents are present at C-13 and C-14 as in friedelanes,[7] fernanes and bauranes, the cracking pattern of the molecule is characteristically changed. It is, therefore, unlikely that wallichiniol has a carbon skeleton analogous to these substances.

The chemical and spectroscopic evidence thus indicate an oleanane or ursane type of carbon framework with a secondary hydroxyl group. Furthermore, the saturated triterpene alcohols so far isolated from nature generally belong to the friedelane or hopane series and the studies in the structure of wallichiniol, which is of biogenetic and chemotaxonomic interest, are in progress.

[5] H. BUDZIKIEWICZ, C. DJERASSI and D. H. WILLIAMS, *Structure Elucidation of Natural Products by Mass Spectrometry*, Vol. 2, p. 137, Holden Day, San Francisco (1964).
[6] H. BUDZIKIEWICZ, C. DJERASSI and D. H. WILLIAMS, *Structure Elucidation of Natural Products by Mass Spectrometry*, Vol. 2, p. 136, Holden Day, San Francisco (1964).
[7] H. BUDZIKIEWICZ, C. DJERASSI and D. H. WILLIAMS, *Structure Elucidation of Natural Products by Mass Spectrometry*, Vol. 2, p. 132, Holden Day, San Francisco (1964).

*Wallichinyl Acetate*

Along with wallichiniol, its acetate was also isolated from the hexane-soluble fraction of the benzene extractive. The acetate $C_{32}H_{54}O_2$, m.p. 315–320°, $[\alpha]_D$ +43°, yielded on alkaline hydrolysis, wallichiniol (cf. mixed m.p. and superimposable i.r. spectra).

*Triterpene Dihydroxy Alcohol*

One of the chromatographic fractions of the 90 per cent alcohol-soluble portion of the benzene extractive gave a small quantity of a triterpene dihydroxy alcohol as silky needles, m.p. 292–296°. It analysed for $C_{30}H_{52}O_2$ and gave positive LB and Nollers tests but a negative test with TNM. The i.r. spectrum of the diol showed an intense band at 3175 $cm^{-1}$ and it formed a monoacetate $C_{32}H_{54}O_3$, m.p. 230°, $[\alpha]_D$ +36°; $\nu_{max}^{KBr}$ 3450, 1150 (tert. OH), 1704 and 1250 $cm^{-1}$ (acetate). Obviously, one of the hydroxyl groups resisting acetylation could be presumed to be tertiary. The m.p. and $[\alpha]_D$ of the diol and the properties of its monoacetate indicated its identity with hopane-3$\beta$,22-diol.[8] However, the i.r. spectrum of the monoacetate when compared with that of 3$\beta$-acetoxy-22-hopanol, showed some variation in the finger-print region (1000–850 $cm^{-1}$) though the mixed m.p. was undepressed. It is quite probable that the diol isolated from *O. wallichii* might be a C-22 epimer of hopane-3$\beta$,22-diol (*lit.* m.p. 284–285°, $\alpha_D$ +53°) reported by Huneck *et al.*

Along with the above triterpenes, $\beta$-sitosterol was isolated from *O. wallichii* rhizomes. The alcohol extractive yielded sucrose.

## EXPERIMENTAL

Optical rotations were measured in 1 per cent $CHCl_3$; m.ps were determined in open capillaries and are uncorrected; i.r. spectra were recorded in KBr films and alumina used for chromatography was neutral Brockmann (E. Merck) quality.

*Isolation of the Constituents*

The plant material was collected from areas adjoining Shillong district (Assam) in September. The shade-dried plant material (7 kg) freed of foreign matter was coarsely powdered and percolated successively with cold benzene (7 l. × 6) and alcohol (7 l. × 5). The concentrated benzene extract was cooled for a few days when a slimy residue separated. The decantate was freed of solvent under reduced pressure and the residue (200 g) thus obtained was partitioned between *n*-hexane (1·5 l.) and alcohol (90%, 1 l.).

The alcohol-soluble fraction was concentrated under reduced pressure, diluted with water and extracted with ether. The ethereal solution was successively shaken with $Na_2CO_3$ (5%, 1·5 l.) and NaOH (3%, 1 l.). The neutral fraction was taken up in ether washed with water, dried and the residue obtained after removal of solvent was dissolved in *n*-hexane and cooled for several days when it gave a viscous greenish residue (14 g). The residue on repeated chromatography over alumina finally yielded hopane-3$\beta$,22-diol (0·2 g) and a triterpene alcohol wallichiniol (330 mg) along with $\beta$-sitosterol (1·8 g).

*Wallichiniol.* The hexane–benzene eluent fractions of the chromatogram on crystallization (hexane) yielded needles, m.p. 310–312°, $[\alpha]_D$+12°, single spot in TLC, $\nu_{max}^{KBr}$ 3450 $cm^{-1}$ (Found: C, 83·62; H, 11·89. $C_{30}H_{52}O$ required: C, 84·10; H, 12·15 per cent). The acetate was obtained as needles (hex:benz), m.p. 315–320°, $[\alpha]_D$+43°; $\nu_{max}^{KBr}$ 1730 and 1230 $cm^{-1}$ (Found: C, 82·17; H, 11·75. $C_{32}H_{54}O_2$ required: C, 81·7; H, 11·5 per cent).

*Oxidation of wallichiniol.* To a mixture of $CrO_3$ (300 mg) in AcOH (5 ml), benzene (1 ml) and water (1 ml) was added a benzene solution (6 ml) of wallichiniol (150 mg) and the reaction mixture shaken for 1 hr with gentle warming and then kept overnight at room temperature. The reaction product on chromatography (alumina, 1:30) yielded a ketone (100 mg), m.p. 285–287°; $\nu_{max}^{KBr}$ 1710 $cm^{-1}$ (Found: C, 84·14; H, 11·92. $C_{30}H_{50}O$ required: C, 84·50; H, 11·73 per cent).

*Huang-Minlon Wolff-Kishner reduction of ketone from wallichiniol.* The above ketone (60 mg) was refluxed with hydrazine hydrate (95 per cent, 1·5 ml) in diethylene glycol (8 ml) and KOH (800 mg) at 175° for 2 hr. The excess of hydrazine hydrate was then distilled off gradually raising the temperature of reaction mixture to 193°. It was again refluxed at the elevated temperature for another 4 hr. The reaction mixture was then

[8] J. Cerny, A. Oystrcil and S. Huneck, *Ber.* **96** (11), 3021 (1963).

cooled, diluted with water, acidified and extracted with ether. The product thus obtained was chromatographed (alumina 1:30) when the hexane eluent fraction yielded a hydrocarbon (9 mg), m.p. 208–211° (Found: C, 87·76; H, 13·04. $C_{30}H_{52}$ required: C, 87·38; H, 12·6 per cent).

*Triterpene dihydroxy alcohol.* It was isolated as fine silky needles from the chromatographic fractions (Bz) of the alcohol-soluble portion of the benzene extract (200 mg), m.p. 292–296°; $\nu_{max}^{KBr}$ 3175 $cm^{-1}$ (Found: C, 80·64; H, 11·99. Calc. for $C_{30}H_{52}O_2$: C, 81·08; H, 11·73 per cent).

*Mono-acetate of triterpene dihydroxy alcohol.* The diol (80 mg), on acetylation, afforded a monohydroxy monoacetate, m.p. 230°; $[\alpha]_D + 36°$; $\nu_{max}^{KBr}$ 3450, 1704 and 1250 $cm^{-1}$ (Found: C, 79·00; H, 11·11 per cent. Calc. for $C_{32}H_{54}O_3$: C, 79·01; H, 11·11 per cent).

*Wallichinyl acetate.* The hexane-soluble portion of the benzene extract was washed free of alcohol, dried and the neutral residue was chromatographed (alumina, 1:15) using *n*-hexane, benzene, chloroform and methanol as eluents, in succession. Repeated chromatography and fractional crystallization yielded, besides wallichiene and wallichienene reported earlier, a triterpene acetate (130 mg) identical with wallichinyl acetate. It was obtained (130 mg) as fine needles (hex:benz), m.p. 315–320°; $[\alpha]_D + 43°$; $\nu_{max}^{KBr}$ 1730, 1250 $cm^{-1}$ (Found: C, 81·81; H, 11·70. $C_{32}H_{54}O_2$ required: C, 81·7; H, 11·5 per cent). It was identical with wallichinyl acetate in regard to its mixed m.p. and i.r. spectrum. On hydrolysis with alcoholic KOH (5 per cent, 10 ml) under reflux for 10 hr, it yielded the alcohol, m.p. 310–312°, $[\alpha] + 12°$; $\nu_{max}^{KBr}$ 3450 $cm^{-1}$; identical with wallichiniol.

*β-Sitosterol.* It was isolated from the hexane as well as the alcohol-soluble portions of the benzene extractive; m.p. and mixed m.p. 137°; $[\alpha]_D - 36°$; acetate, m.p. and mixed m.p. 128°; $[\alpha]_D - 37°$. The i.r. spectra of β-sitosterol and its acetate were superimposable with those of the authentic samples.

*Sucrose.* The alcoholic percolate of the rhizomes when concentrated and kept overnight deposited sucrose (40 g). It was recrystallized with alcohol (90 per cent), m.p. 184–185°, $[\alpha]_D + 67°$ (Found: C, 43·02; H, 6·80. Calc. for $C_{12}H_{22}O_{11}$: C, 42·11; H, 6·42 per cent). On hydrolysis with HCl (5 per cent) it gave glucose and fructose.

*Acknowledgements*—The authors thank Professor S. Huneck, University of Dresden, for authentic samples of 3β-acetoxy, hopan-22-ol and hopane-3β,22-diol, Mr. J. Saran for microanalyses and Dr. L. B. Singh, Director, National Botanic Gardens, for interest in this investigation.