FUROQUINOLINE AND PYRANO-2-QUINOLONE ALKALOIDS OF *VEPRIS STOLZII*[1]

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ABSTRACT.—Six alkaloids and the pentacyclic triterpene lupeol were isolated from the stem bark of *Vepris stolzii* Verdoorn (Rutaceae). Three of the alkaloids were identified as the furoquinolines skimmianine (**1**) and gamma-fagarine (**3**) and the pyrano-2-quinolone veprisine (**2**) by comparison with authentic samples or literature data. The remaining three alkaloids, all pyrano-2-quinolones, were novel. They were identified, on the basis of spectral data and positive Gibbs tests on their hydrolysis products, as *N*-methyl-8-(3",3"-dimethylallyloxy)-flindersine (**4**), *N*-methyl-7-methoxy-8-(3",3"-dimethylallyloxy)-flindersine (**5**), and *N*-methyl-7-methoxy-8-(2",3"-epoxy-3",3"-dimethylallyloxy)-flindersine (**7**). The chemotaxonomic significance of these alkaloids was discussed.

The African species of the sub-family Toddalioideae (Rutaceae) have proved (1–5) to be a good source of the furoquinoline, pyranoquinolone and acridone alkaloids that typify the family as a whole (6). Limonoids have also been found in a number of species (1, 5, 7, 8), but coumarins have not so far been recorded from any species other than the atypical *Toddalia aculeata* (9). As part of our continuing study of the distribution of secondary compounds in this group (1, 2, 8), we have investigated the stem bark of *Vepris stolzii*, a small tree of montane forest, indigenous to east Africa (10, 11). No previous phytochemical examination of this species has been reported.

RESULTS

Column chromatography of the petroleum spirit extract of the stem bark yielded the pentacyclic triterpene lupeol and six furoquinoline and pyranoquinolone alkaloids. Lupeol, skimmianine (**1**) and veprisine (**2**) were identified by direct comparison with authentic samples (1). A third alkaloid, *gamma*-fagarine (**3**), was identified by comparison of spectral data with that published (12, 13).

The remaining three alkaloids, which were all isolated as oils, exhibited the spectroscopic characteristics of *N*-methylpyrano-2-quinolones. Thus, their complex uv spectra showed no shift on addition of HCl, and the ir spectra had carbonyl absorption at 1640 cm^{-1}, typical of 2-quinolones (14, 15). Their ^1H nmr spectra exhibited a sharp singlet (6H) at δ 1.50 together with an AB quartet centred at δ 5.50 and 6.75, typical of a 2,2-dimethylpyran system, and a further singlet (3H) at about δ 3.78 for N–Me. The remainder of the ^1H nmr spectra of the three alkaloids varied, indicating that they differed in substitution of the benzene ring.

The simplest had a MW of 325, shown by exact mass measurement to be $C_{20}H_{23}NO_3$. Eims showed facile loss of *m/z* 68 ($C_5H_8^+$) indicative of a prenyloxy side-chain. This was confirmed by the ^1H nmr spectrum which exhibited resonances at δ 1.80 and 1.83 (3H each), 4.64 (2H) and 5.53 (1H) typical of a 3,3-dimethylallyloxy substituent. The remaining resonances were for three aromatic protons which, from their coupling interactions, had to be adjacent to each other. One of the aromatic protons occurred as a double doublet at δ 7.87, its highly deshielded position being typical of H–5 (2, 3). After hydrolysis, the alkaloid gave a positive Gibbs test for a phenol with a free *para*-H. On the basis of the above, the alkaloid was identified as *N*-methyl-8-(3",3"-dimethylallyloxy)-flindersine (**4**), which appears to be novel.

The second novel alkaloid had a MW of 355, shown by exact mass measurement

[1]Paper 15 in the series "Chemosystematics in the Rutaceae." For paper 14 see W. E. Campbell, G. J. Provan and P. G. Waterman, *Phytochemistry*—in press.

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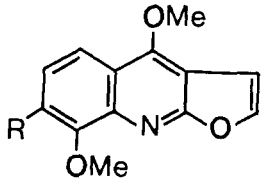
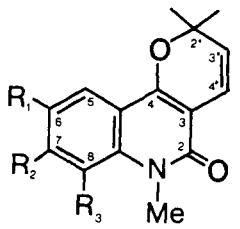
to be $C_{21}H_{25}NO_4$. The ^1H nmr spectrum differed from that of (**4**) by showing only two aromatic protons, as an AB quartet exhibiting *ortho*-coupling, and an additional OMe resonance at δ 3.93. The strongly deshielded position of one of the aromatic protons (δ 7.70) required that it be assigned to C–5 permitting the assignment of either structure **5** or structure **6** to this compound. A Gibbs test on the hydrolysis product proved positive indicating a free *para*-H with respect to the *O*-prenyl substituent of the original alkaloid and confirming its identification as *N*-methyl-7-methoxy-8-(3",3"-dimethylallyloxy)-flindersine (**5**).



1 R = OMe

3 R = H



2 $R_2 = R_3 = OMe$, $R_1 = H$

4 $R_3 = OCH_2\overset{1'''}{C}H{:}\overset{2'''}{C}\overset{3'''}{(Me)_2}$, $R_1 = R_2 = H$

5 $R_3 = OCH_2CH{:}C(Me)_2$, $R_2 = OMe$, $R_1 = H$

6 $R_2 = OCH_2CH{:}C(Me)_2$, $R_3 = OMe$, $R_1 = H$

7 $R_3 = OCH_2CH\text{—}C(Me)_2$ (O), $R_2 = OMe$, $R_1 = H$

8 $R_1 = R_2 = OMe$, $R_3 = H$

9 $R_2 = OMe$, $R_1 = R_3 = H$

The third alkaloid had a MW of 371, shown by exact mass measurement to be $C_{21}H_{25}NO_5$. The ^1H nmr spectrum was identical to **5** except for the signals for the *O*-prenyl side-chain in which both Me and H–2" resonances showed considerable shielding. These changes are in accord with requirements (16) for 2,3-epoxidation of a 3,3-dimethylallyl side-chain and the presence of the oxygenated substituent was confirmed by facile loss of *m/z* 84 ($C_5H_8O^+$) in the eims. A positive Gibbs test on the hydrolysis product confirmed a substitution pattern identical to **5** and permitted the alkaloid to be identified as *N*-methyl-7-methoxy-8-(2",3"-epoxy-3",3"-dimethylallyloxy)-flindersine (**7**).

DISCUSSION

Six species of *Vepris* have now been reported to contain alkaloids; *V. stolzii*, *V. louisii* (3–5) and *V. heterophylla* (17) from Africa, and *V. ampody* (18), *V. bilocularis* (18) and *V. pilosa* (19) from Malagasy and the Indian sub-continent.

All six species produce quinoline alkaloids, but only the latter three have so far been reported to produce acridones. According to Gilbert (20) *V. louisii* is most closely allied to *V. stolzii*. The close similarity between the alkaloids of these two taxa, they are the only two species of *Vepris* to produce pyrano-2-quinolones and in both substitution is restricted to C–7 and C–8, bears out this contention. However, *V. louisii* has also yielded limonin (5), and this could not be detected in *V. stolzii*.

Pyrano-2-quinolones are rare in the African Toddalioideae. The only other sources so far reported are the *Oricia* species, *O. suaveolens* (1), which has yielded oricine **8**, and *O. renieri* (2), which has yielded **2**, **8** and *N*-methyl-7-methoxy-flindersine (**9**). In addition, both *Oricia* species have yielded acridones. In pyrano-2-quinolones from both *Oricia* species substitution at C–6 occurs as well as at C–7 and C–8. However, whilst this distinction holds at present for pyrano-2-quinolones it should be noted that C–6 substitution occurs among the furo-quinolines of all of the other four *Vepris* species.

EXPERIMENTAL[3]

PLANT MATERIAL.—The stem bark of *Vepris stolzii* Verdoorn was collected in montane forest at 2300 m from near Gisovu in the prefecture of Kibuye, Rwanda, in February 1980. A voucher specimen (D. Bridson 440) has been deposited at the herbarium of the Royal Botanic Gardens, Kew, England.

EXTRACTION AND ISOLATION OF THE ALKALOIDS.—Ground stem bark (300 g) was extracted with petroleum spirit (bp 40–60°) and then chloroform. The petroleum spirit extract was concentrated and yielded a solid (3 g) which, after recrystallization from petroleum spirit/ethyl acetate, was identified as lupeol (mixed mp, ir, or, tlc). Concentration of the chloroform extract gave a brown oil (7 g), which was shown by tlc (silica gel, solvent A) to contain six blue fluorescent spots that gave positive reactions for alkaloids. The oil was subjected to cc over silica gel. Elution of the column with petroleum spirit (bp 60–80°) containing 15% ethyl acetate gave 17 mg of *N*-methyl-7-methoxy-8-(3",3"-dimethylallyloxy)-flindersine (**5**) followed by 45 mg of veprisine, **2**, (mixed mp, ^{1}H nmr, eims, uv, ir, tlc). Further elution with petroleum spirit containing 18% ethyl acetate gave a mixture of two compounds which were separated by plc on silica gel (solvent B) to give 25 mg of *N*-methyl-8-(3",3"-dimethylallyloxy)-flindersine, **4**, (R_f 0.40) and 18 mg of N-methyl-7-methoxy-8-(2",3"-epoxy-3",3"-dimethyl-allyloxy)-flindersine, **7**, (R_f 0.27). Final elution with petroleum spirit containing 35% ethyl acetate gave a mixture of the remaining two alkaloids, which were separated by plc on silica gel (solvent A) to give 32 mg of *gamma*-fagarine, **3**, (R_f 0.17) mp 138° (^{1}H nmr, eims, uv, ir), lit. (12) 138°, and 47 mg of skimmianine, **1**, (R_f 0.10), (mixed mp, ^{1}H nmr, eims, uv, ir, tlc).

IDENTIFICATION OF NOVEL ALKALOIDS.—*N*-methyl-8-(3",3"-dimethylallyloxy)-flindersine (**4**) was an oil showing the following: Eims (*m/z*, rel intensity): 325(47), 310(2), 257(52), 242(100), 213(12); exact mass 325.1680, calc for $C_{20}H_{23}NO_3$: 325.1678; uv λ max (EtOH) 235, 262, 270, 320sh, 338, 352, 366; +HCl—no change; ir γ max ($CHCl_3$) 1640, 1620, 1600; ^{1}H nmr (90 MHz, $CDCl_3$): 1.50 (s, 6H, 2'-$(CH_3)_2$), 1.80, 1.83 (2 x s, 2 x 3H, 3"-$(CH_3)_2$), 3.74 (s, 3H, N-CH_3), 4.64 (d, 2H, *J* 7, 1"-CH_2), 5.53 (t, 1H, *J* 7, 2"-CH), 5.48, 6.73 (AB quartet, 2H, *J* 10, 3'-H and 4'-H), 6.84 (dd, 1H, *J* 9 and 8, 6-H), 6.88 (dd, 1H, *J* 8 and 2, H-7), 7.87 (dd, 1H, *J* 9 and 2, H-5). A solution of the alkaloid in acetic acid and H_2SO_4 was heated to 80° for 30 min to effect hydrolysis. The hydrolysis product was dissolved in $H_3BO_3/Na_2B_4O_7$ buffer (pH 9) and reacted with a saturated solution of 2,6-dichloro-*p*-benzoquinone-4-chloramine in water. A blue color developed indicative of a positive Gibbs test.

N-methyl-7-methoxy-8-(3",3"-dimethylallyloxy)-flindersine (**5**) was an oil showing the following: Eims (*m/z*, rel intensity): 355(9), 338(1), 303(14), 287(39), 272(100), 257(13), 250(32), 221(4), 206(1); exact mass 355.1789, calc for $C_{21}H_{25}NO_4$: 355.1783; uv λ max (EtOH) 232, 260sh, 270sh, 316sh, 326, 351, 366; + HCl—no change; ir γ max ($CHCl_3$) 1640, 1600, 1580; ^{1}H nmr (90 MHz, $CDCl_3$): 1.49 (s, 6H, 2'-$(CH_3)_2$), 1.73 (broad s, 6H, 3"-$(CH_3)_2$), 3.78 (s, 3H, N-CH_3), 3.93 (s, 3H, O-CH_3), 4.68 (d, 2H, *J* 7, 1"-CH_2), 5.52 (t, 1H, *J* 7, 2"-CH), 5.48, 6.73 (AB quartet, 2H, *J* 10, 3'-H and 4'-H), 6.83, 7.70 (AB quartet, 2H, *J* 9, 6-H and 5-H). The hydrolysis product gave a positive Gibbs test.

N-methyl-7-methoxy-8-(2",3"-epoxy-3",3"-dimethylallyloxy)-flindersine (**7**) was an oil showing the following: Eims (*m/z*, rel intensity): 371(85), 356(100), 314(2), 305(1), 286(19), 272(73), 257(17); exact mass 371.1718 calc for $C_{21}H_{25}NO_5$: 371.1733; uv λ max (EtOH) 235, 263, 273, 315sh, 336, 349, 368; ir γ max ($CHCl_3$) 1640, 1610, 1595; ^{1}H nmr (90 MHz, $CDCl_3$): 1.38, 1.40 (2 x s, 2 x 3H, 3"-$(CH_3)_2$), 1.49 (s, 6H, 2'-$(CH_3)_2$), 3.21 (t, 1H, *J* 5, 2"-CH), 3.82 (s, 3H, N-CH_3), 3.93 (s, 3H, O-CH_3), 4.26 (d, 2H, *J* 5, 1"-CH_2), 5.50, 6.75 (AB quartet, 2H, *J* 10, 3'-H

[3]Melting points were determined by means of a Kofler hot plate and are uncorrected. ^{1}H nmr spectra were run on a Perkin-Elmer R.32 instrument. Eims (probe) were obtained at elevated temperature and 70 eV. For tlc: solvent A refers to toluene:ethyl acetate-formic acid (5:4:1) and solvent B to benzene:ethyl acetate (3:1).

and 4'-H), 6.93, 7.72 (AB quartet, 2H, *J* 9, 6-H and 5-H). The hydrolysis product gave a positive Gibbs test.

ACKNOWLEDGMENTS

The supply of plant material by Dr. D. Bridson, Royal Botanic Gardens, Kew, is gratefully acknowledged. One of us (S.A.K.) wishes to thank the University of Khartoum for the award of a scholarship. This study was supported by an equipment grant (no. A 80754) from the Science Research Council.

Received 14 September 1981

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