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PYRANOQUINOLONES AND ACRIDONES FROM *VEPRIS BILOCULARIS*

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Key Word Index—*Vepris bilocularis*; Rutaceae–Toddalioideae; leaves; quinolines; pyrano-2-quinolones; acridones; alkaloids.

Abstract—Besides already known furoquinolones and acridones, three new pyrano-2-quinolones and one new acridone were isolated from the leaves of south Indian *Vepris bilocularis*. The novel alkaloids were identified by spectroscopic means and named 7-methoxyflindersine, 7-prenyloxyflindersine, *N*-methyl-7-prenyloxy-flindersine and vebilocine.

INTRODUCTION

In connection with our current screening for biologically active compounds [1–3], we have investigated the lipophilic leaf extract of the south Indian *Vepris bilocularis*. This rare plant is a medium- to large-sized tree growing in evergreen forests [4]. Previous phytochemical investigations of Indian collections revealed the presence of acridones mainly substituted at the C-1, C-2 and C-3 position (arborinine-type), together with different furoquinolines [5, 6]. The present results obtained from a collection originating from the Peechi forest, a natural reserve in Kerala near Trichur [7], differ, accumulating pyrano-2-quinolones (**1–4**) and a 3-prenyloxy acridone (**5**). From the seven compounds isolated, four have shown to be hitherto undescribed alkaloids.

RESULTS AND DISCUSSION

Analytical HPLC–UV diode array analysis of the chloroform phase of a methanolic leaf extract showed the characteristic ultraviolet spectra of a series of quinolones and acridones. The spectra of quinolones **1–3** resemble those of the already known *N*-methylflindersine [8, 9] and *N*-methyl-7-methoxyflindersine [10], whereas the similar spectrum of quinolone **4** is shifted somewhat to higher wavelengths. The latter compound was identified by ^{1}H NMR as the previously described 6-methoxyflindersine (haplamine, **4**) [11].

The IR spectra of the unknown compounds **1–3** showed three significant bands in the region between 1655 and 1600 cm^{-1}, which are typical of 2-quinolinones [9, 12]. Compounds **1** and **2** were characterized by N–H stretching vibrations at 3414 cm^{-1}. The ^{1}H NMR spectra of all derivatives **1–4** showed the well-known pattern of a pyran ring system condensed to an aromatic system, with typical resonances for the geminal methyl groups (singlet of 6 H at δ *ca* 1.5) and the olefinic protons (AB system at δ *ca* 5.5 and 6.75 with $J = ca$ 10 Hz). The signal pattern of the three remaining aromatic resonances (chemical shifts and coupling constants) are essentially the same for compounds **1–3**. One aromatic position at the non-hetero ring of the quinolinone moiety is occupied with a methoxyl group in the case of **1** and with prenyloxy substituents in **2** and **3**. Compound **3** differs from **2** only by *N*-methylation. The basic structures of a methoxyflindersine for **1**, a prenyloxyflindersine for **2** and *N*-methyl-prenyloxyflindersine for **3** were also supported by mass spectral data with the corresponding $[M]^+$.

Following from the coupling pattern of the aromatic protons, the positions of the alkyloxy groups should be either at C-6 or C-7. Comparison of the chemical shifts of the aromatic resonances for **1–3** with the corresponding data for the unsubstituted angular pyranoquinolinone flindersine [9] and its 6-methoxy derivative (**4**) [11], as well as the NOE difference data for **3**, showed that only 7-methoxy and 7-prenyloxy derivatives were consistent with all available data.

The most characteristic aromatic proton of the non-hetero ring in the angular pyrano-2-quinolinone system is the lowest field proton at position 5. In flindersine, which carries no further substituents at ring A, the chemical shift of 5-H is 7.89 [9], in haplamine with a methoxy substituent at C-6 the shift of 5-H is 7.25 (*d* with *meta*-coupling of 2.6 H, no *ortho*-coupling) [11]. In our series of compounds **1–3**, this significant low-field resonance is found at δ 7.77 (*d*, with an *ortho*-coupling of $J = 9.4$ Hz) for **1**, δ 7.78 (*d*, $J = 8.9$ Hz) for

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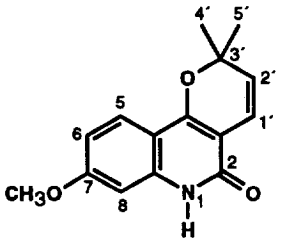
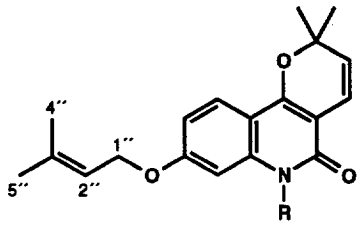
2, and δ 7.86 (*d*, $J = 8.8$ Hz) for **3**. This implies that the alkyloxy substituents are positioned at C-7. This agrees also with the other aromatic resonances: *ortho–meta*-coupling *dd*s for 6-H at δ 6.78 ($J = 9.4$ and 2.3 Hz) for **1**, δ 6.79 ($J = 8.9$ and 2.1 Hz) for **2**, and δ 6.82 ($J = 8.8$ and 2.2 Hz) for **3**, and *meta*-coupled doublets for 8-H at δ 6.80 ($J = 2.3$ Hz) for **1**, δ 6.70 ($J = 2.1$ Hz) for **2**, and δ 6.76 ($J = 2.2$ Hz) for **3**. These assignments, following from simple considerations of chemical shifts and coupling behaviour, were unambiguously confirmed by NOE difference measurements on **3**, showing all expected NOEs for the 7-alkyloxy substituted system, from the prenyl methylene group 1″-H_2 to the aromatic 6-H and 8-H and from *N*-methyl to 8-H. An additional NOE from 1″-H_2 to the *cis*-methyl group 4″-H_3 allowed the assignment of the two prenylic methyl groups (see Experimental). The compounds were named as 7-methoxyflindersine (**1**), 7-prenyloxyflindersine (**2**), and *N*-methyl-7-prenyloxyflindersine (**3**), according to the commonly used semi-trivial names for flindersine derivatives (cf. ref. [13]).

In addition to this series of pyranoquinolones (**1–4**), the leaf extract of *V. bilocularis* was characterized by large amounts of acridones. The main compound is 1,2,3-trimethoxy-*N*-methylacridone (**6**), which is accompanied by arborinine (**7**), a widespread acridone within the Rutaceae [6]. A further unknown, less polar substance also showed the typical UV pattern of acridones [14] with maxima at 387, 323, 296, 272, 262, 250 and 225 nm. The IR spectrum of this compound showed the C=O stretching band at 1632 cm^{-1} and further signals at 1596 and 1562 cm^{-1} confirming the acridone skeleton [14]. The ^{1}H NMR spectrum showed a sharp low-field singlet of 1H at δ 14.83, which is typical for 1-OH with a hydrogen bridge to the 9-carbonyl group of acridones. Further substituents which could be identified in the proton spectrum were *N*-Me (δ 3.79, *s*, 3H) and the characteristic signals for a prenyloxy side-chain. The mass spectral data with $[M]^+$ of *m/z* 309 (15%) and a base peak at *m/z* 241 (100%, corresponding to phenol after loss of prenyl) confirm this basic structure. The only remaining question was the position of the prenyloxy side-chain. One aromatic ring of the acridone system was without any substituent (four protons with characteristic coupling pattern) and the two remaining aromatic protons of the other aromatic ring showed no *ortho*-coupling. They appeared as a very narrow *meta*-coupled AB system at rather high-field (δ 6.36 and 6.35, $J = 2.0$ Hz). This is only possible for 1-hydroxy-3-prenyloxy-substitution, explaining the coupling pattern and the high-field shift due to a two-fold influence of the oxygen substituents. Therefore, the structure of a 1-hydroxy-*N*-methyl-3-prenyloxy-9-acridone was assigned to compound **5**, designated as vebilocine.

Whereas the accumulation of prenylated quinolones seems to be a common biogenetic trend in the genus *Vepris*, the formation of acridones is confined to five from nine species [6, 15, 16]. A similar chemical profile characterizes the African genus, *Teclea*, supporting the close relationship with *Vepris* [17]. The exclusive oxygenation at position C-7 of the quinoline moiety of compounds **1–3** is remarkable since it has only been observed so far in African *Oricia*, which again is a member of the subfamily Toddalioideae [10, 15]. However, in a chemosystematic report of the African Toddalioideae [15], 8-prenyloxy-*N*-methylflindersine from *V. stolzii* [12] was erroneously depicted as the

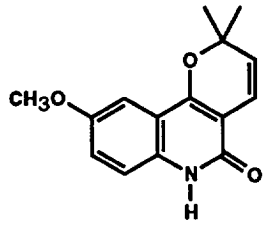


1

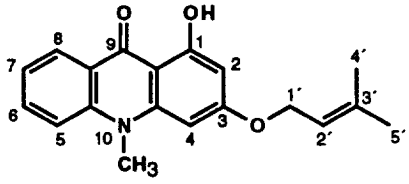


2 R = H

3 R = CH_3



4



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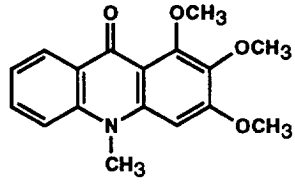

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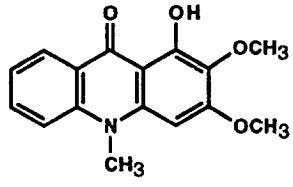

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7-prenyloxy isomer by exchange of positions 7 and 8 in a series of 8-prenyloxy-substituted angular pyranoquinolones.

EXPERIMENTAL

General. NMR: 400 and 250 MHz, $CDCl_3$. HPLC: UV diode-array detection, 230 nm, column 290 × 4 mm (Spherisorb ODS, 5 μm), mobile phase MeOH (gradient 60–100%) in aq. buffer (0.015 M H_3PO_4–0.0015 M NBu_4OH, pH 3), flow rate 1 ml min^{-1}.

Plant material. Twigs from *V. biloculars* (Wight et Arn.) Engl. were collected in Peechi Forest, Kerala, India, 23 February 1991. Voucher specimens are deposited at the Herbarium of the University of Vienna.

Extraction and isolation. Fresh leaves (dry wt 95 g) were coarsely chopped in the field and directly preserved in MeOH. After shipping back to Vienna, the MeOH ext. was filtered and concd. The $CHCl_3$ fr. from the aq. soln was evapd to dryness (1200 mg) and roughly sepd by CC (Merck silica gel 60, 35–70 mesh). The frs eluted with 50% Et_2O in petrol, Et_2O and 5% MeOH in Et_2O, contained the described compounds. The combined frs (400 mg) were sepd by prep. MPLC with 10, 30 and 50% (v/v) EtOAc in petrol (400 × 38 mm column, Merck LiChroprep silica gel 60, 25–40 μm, UV detection, 254 nm). MPLC with 10% EtOAc afforded 9 mg **5**, 30% EtOAc yielded 40 mg **3**, whereas 50 mg **2**, 58 mg **1** and 62 mg **4** were eluted with 50% EtOAc as crude products. Compounds **1** (30 mg), **2** (28 mg), **4** (15 mg) and **5** (5 mg) were obtained by recrystallization from Et_2O–petrol, while **3** (20 mg) was purified by repeated MPLC. Further CC frs eluted with 10 and 25% MeOH in Et_2O yielded the known compounds, **6** (120 mg, pure) and **7** (50 mg after recrystallization from Et_2O).

7-Methoxyflindersine {5,6-*dihydro*-8-*methoxy*-2,2-*dimethyl* - 2H - *pyrano*[3,2-c]*quinolin* - 5 - *one*} (**1**). Crystals, mp 194–196°. UV λ^{Et_2O} nm: 374, 370 sh, 356, 340, 327 sh, 318 sh, 304 sh, 264 sh, 233, 223. IR ν^{CCl_4} cm^{-1}: 3414 vw, 3156 w, 3082 w, 2978 m, 2936 m, 2868 w, 2838 w, 2818 w, 1654 s, 1622 s, 1602 m, 1564 m, 1508 m, 1492 m, 1462 w, 1444 w, 1418 m, 1376 w, 1362 m, 1356 m, 1336 w, 1264 m, 1220 m, 1186 m, 1176 m, 1166 w, 1130 m, 1120 m, 1104 w, 1038 w, 972 w, 884 m, 840 w, 716 w, 700 w, 680 w, 664 w. ^{1}H NMR ($CDCl_3$, 250 MHz): δ 11.70 (*br s*, 1H, N-H), 7.77 (*d*, 1H, J = 9.4 Hz, 5-H_{Ar}), 6.78 (*dd*, 1H, J = 9.4, 2.3 Hz, 6-H_{Ar}), 6.80 (*d*, 1H, J = 2.3 Hz, 8-H_{Ar}), 6.74 (*d*, 1H, J = 9.9 Hz, 1′-H), 5.49 (*d*, 1H, J = 9.9 Hz, 2′-H), 3.90 (*s*, 3H, 7-OMe), 1.52 (*s*, 6H, 4′-H_3, 5′-H_3). MS (70 eV, 80°) *m/z* (rel. int.): 257 (88) [M^+, $C_{15}H_{15}NO_3$], 242 (100), 228 (17), 213 (12), 199 (59), 121 (35), 106 (17); HR MS: $C_{15}H_{15}NO_3$, $[M]^+$ 257.1052, calc. 257.1052.

7-Prenyloxyflindersine {5,6 - *dihydro* - 8 - (3 - *methylbutyloxy*) - 2,2 - *dimethyl* - 2H - *pyrano*[3,2-c]*quinolin*-5-*one*} (**2**). Crystals, mp 165–167°. UV λ^{Et_2O} nm: 374, 370 sh, 356, 341, 328 sh, 318 sh, 304 sh, 262, 234, 224. IR ν^{CCl_4} cm^{-1}: 3414 vw, 3160 w, 3084 w, 2978 m, 2934 m, 2898 w, 2812 w, 1652 s, 1620 s, 1602 m, 1562 w, 1506 m, 1490 m, 1462 w, 1418 m, 1378 w, 1356 m, 1336 w, 1278 w, 1258 m, 1242 w, 1208 m, 1184 m, 1164 w, 1130 m, 1120 m, 1102 w, 1014 w, 990 w, 962 w, 886 w, 842 w, 700 w, 672 w, 662 w. ^{1}H NMR ($CDCl_3$, 250 MHz): δ 10.90 (*br s*, 1H, N-H), 7.78 (*d*, 1H, J = 8.9 Hz, 5-H_{Ar}), 6.79 (*dd*, 1H, J = 8.9, 2.1 Hz, 6-H_{Ar}), 6.71 (*d*, 1H, J = 9.9 Hz, 1′-H), 6.70 (*d*, 1H, J = 2.1 Hz, 8-H_{Ar}), 5.50 (*t sept*, 1H, J = 6.8, <1 Hz, 2″-H), 5.48 (*d*, 1H, J = 9.9 Hz, 2′-H), 4.60 (*br d*, 2H, J = 6.8 Hz, 1″-H), 1.82 (*br s*, 3H, 5″-H_3), 1.79 (*br s*, 3H, 4″-H_3), 1.51 (*s*, 6H, 4′-H_3, 5′-H_3). MS (70 eV, 120°) *m/z* (rel. int.): 311 (4) [M^+, $C_{19}H_{21}NO_3$], 287 (13), 272 (40), 257 (11), 243 (13), 228 (59), 167 (18), 149 (100), 83 (37), 69 (45); HR MS: $C_{19}H_{21}NO_3$ $[M]^+$ 311.1521 calc. 311.1521.

7-Prenyloxy-N-*methylflindersine* {5,6-*dihydro*-8-(3-*methylbutyloxy*) - 2,2,6 - *trimethyl* - 2H - *pyrano*[3,2 - c]*quinolin*-5-*one*} (**3**). Oil. UV λ^{Et_2O} nm: 375, 372 sh, 357, 341, 328 sh, 305 sh, 267, 235, 226 sh. IR ν^{CCl_4} cm^{-1}: 3058 w, 2976 m, 2932 m, 2874 w, 2874 w, 2856 w, 1648 s, 1610 s, 1600 m (sh), 1564 m, 1514 m, 1464 m, 1446 m, 1418 m, 1382 w, 1362 m, 1328 m, 1296 w, 1266 w, 1230 s, 1186 m, 1154 m, 1134 w, 1126 w, 1086 m, 1014 m, 986 w, 946 w, 886 w, 842 w, 668 w, 662 w. ^{1}H NMR ($CDCl_3$, 250 MHz): δ 7.86 (*d*, 1H, J = 8.8 Hz, 5-H_{Ar}), 6.82 (*dd*, 1H, J = 8.8, 2.2 Hz, 6-H_{Ar}), 6.76 (*d*, 1H, J = 2.2 Hz, 8-H_{Ar}), 6.73 (*d*, 1H, J = 9.9 Hz, 1′-H), 5.51 (*t sept*, 1H, J = 6.8, *ca* 1.4 Hz, 2″-H), 5.47 (*d*, 1H, J = 9.9 Hz, 2′-H), 4.62 (*d*, 2H, J = 6.8 Hz, 1″-H), 3.64 (*s*, 3H, N-Me), 1.82 (*br s*, 3H, 5″-H_3), 1.78 (*br s*, 3H, 4″-H_3), 1.49 (*s*, 6H, 4′-H_3, 5′-H_3). NOE-difference expts: irradiation at δ 4.62 (1″-H_2) resulted in strong signal enhancements at δ 6.82 (6-H), 6.76 (8-H), 1.78 (4″-H_3); irradiation at δ 3.64 (*N*-Me) showed a strong effect at δ 6.76 (8-H). MS (70 eV, 140°) *m/z* (rel. int.): 325 (14) [M^+, $C_{20}H_{23}NO_3$], 257 (25), 242 (100), 213 (6), 199 (2), 149 (3), 69 (10).

1-Hydroxy-N-*methyl*-3-*prenyloxyacridone* (**5**). Yellow crystals, mp 145–147°. UV λ^{Et_2O} nm: 387, 323, 296, 272, 262, 250, 225. IR ν^{CCl_4} cm^{-1}: 2978 w, 2934 w, 2926 w, 1632 s, 1596 s, 1562 m, 1508 w, 1468 m, 1428 w, 1416 w, 1382 w, 1356 w, 1312 m, 1280 m, 1226 m, 1200 w, 1172 m, 1158 m, 1092 w, 1034 w, 1012 w, 930 w, 878 w, 672 w. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 14.83 (*s*, 1H, 1-OH), 8.48 (*dd*, 1H, J = 8.0, 1.7 Hz, 8-H), 7.73 (*ddd*, 1H, J = 8.8, 7.1, 1.7 Hz, 6-H), 7.49 (*br d*, 1H, J = 8.8 Hz, 5-H), 7.30 (*ddd*, 1H, J = 8.0, 7.1, 0.9 Hz, 7-H), 6.36 and 6.345 (two *d*, narrow AB-system, 2H, J = 2.0 Hz, 2-H, 4-H), 5.52 (*t sept*, 1H, J = 6.8, *ca* 1.3 Hz, 2′-H), 4.63 (*d*, 1H, J = 6.8 Hz, 1′-H), 3.79 (*s*, 3H, N-Me), 1.83 (*br s*, 3H, 5′-H), 1.79 (*br s*, 3H, 4′-H). MS (70 eV, 160°) *m/z* (rel. int.): 309 (15) [M^+, $C_{19}H_{19}NO_3$], 294 (2), 266 (5), 241 (100), 213 (12), 184 (8), 97 (7), 69 (14).

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