Phytochemistry, Vol. 28, No. 5, pp. 1517–1519, 1989.
Printed in Great Britain.

0031-9422/89 $3.00 + 0.00

QUINOLONE AND CARBAZOLE ALKALOIDS FROM *CLAUSENA ANISATA*

BONAVENTURE TCHALEU NGADJUI, J. FOYERE AYAFOR, BEIBAM L. SONDENGAM and JOSEPH D. CONNOLLY*

Department of Organic Chemistry, Faculty of Science, The University of Yaounde, Box 812, Yaounde, Cameroon; *Chemistry Department, The University, Glasgow G12 8QQ, Scotland, U.K.

(*Received in revised form* 3 *August* 1988)

Key Word Index—*Clausena anisata*; Rutaceae; stem bark and roots; alkaloids; 2-quinolones and carbazoles.

Abstract—From the combined stem bark and root extracts of *Clausena anisata* we isolated two new alkaloids, 1-methyl-3,4-dimethoxy-2-quinolone and 3-formyl-1-hydroxycarbazole, and four known alkaloids identified as heptaphylline, girinimbine, ekeberginine and 3-methylcarbazole. The structure of the new alkaloids were assigned on the basis of ^{1}H and ^{13}C NMR evidence.

INTRODUCTION

The family Rutaceae which comprises more than 140 genera distributed in 1300 species [1] is known to contain alkaloids of complex structural diversity [2, 3]; a number of carbazole alkaloids have been isolated [4]. The isolation of mupamine (**1**) [5, 6] and clausanitin (**2**) [7] from the roots and atanisatin (**3**) [7] from the stem bark of *Clausena anisata* (Will) has been reported. In continuation of our own studies on *C. anisata* [8], we have isolated from the combined extracts of its stem bark and roots a new carbazole alkaloid, for which we propose the name *O*-demethylmurrayanine, along with four known compounds. A second novel alkaloid, a 2-quinolone, *N*-methylswietenidine-B, was also characterized. We now report the structural elucidation of these new alkaloids.

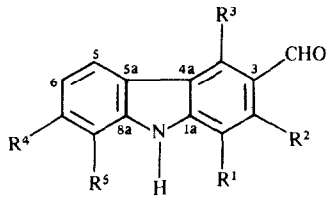
RESULTS AND DISCUSSION

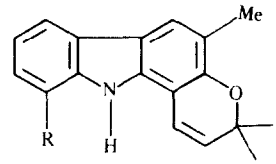
The petrol and chloroform extracts of the combined stem bark and roots of *C. anisata* afforded a mixture of sterols, 10 substituted coumarins, six limonoids [8] and six alkaloids upon successive chromatography on silica gel and recrystallizations. Four of the six alkaloids were identified as heptaphylline (**4**) [9], ekeberginine (**5**) [10], girinimbine (**7**) [11] and 3-methylcarbazole (**8**) [12] by comparison of their physical and spectral data with those published.

The first new alkaloid, *O*-demethylmurrayanine (**6**), mp 237–238°, analysed for $C_{13}H_9NO_2$ ($[M]^+$, *m/z* 211). Preliminary colour tests, a green colouration with a mixture of concentrated sulphuric acid and nitric acid and a blue green colouration with an ethanolic ferric chloride solution, showed that **6** was a carbazole bearing a phenolic hydroxyl group [13]. The presence of a hydroxyl group was further confirmed by strong adsorption at ν_{max} 3460 cm^{-1} in the IR spectrum. The IR spectrum also showed a band at ν_{max} 1670 cm^{-1} indicating the presence of a conjugated aldehyde group. The UV spectrum (see Experimental) was typical of that of 3-formyl carbazoles [13, 14]. The ^{1}H NMR spectrum of **6** was well-resolved and exhibited two doublets of one-proton each at δ_H 8.15 (J = 1.4 Hz) and 7.33 (J = 1.4 Hz). The values of their chemical shifts indicated that they were H-4 and H-2, respectively, of a substituted carbazole [7, 9]. The presence of an aldehyde group was shown by the sharp singlet at δ_H 9.89 (1H) and the formation of a dinitrophenyl hydrazone derivative. Furthermore, the ^{1}H NMR spectrum showed an *ortho*-disubstituted benzene ring (Table 1). The above data are consistent with the presence of a carbazole moiety with aldehyde and hydroxyl substituents on one of the rings and suggest structure (**6**) for *O*-demethylmurrayanine. The ^{13}C NMR spectral data (Table 1) of *O*-demethylmurrayanine were fully assigned and are in perfect accordance with structure (**6**).

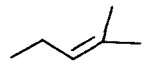
The second new alkaloid (**9**), $C_{12}H_{13}NO_3$, was optically inactive and not phenolic. Its UV spectrum (see Experimental) in neutral and in acid solution was consistent with that of a 2-quinolone [15]. This was confirmed by the IR absorption at ν_{max} 1630 cm^{-1} (2-quinolone carbonyl), and a ^{13}C NMR signal (Table 2) at δ_C 161.2 for the 2-quinolone carbonyl carbon [16]. The ^{1}H NMR spectrum of **9** was almost identical in the downfield region with that of swietenidine-B (**10**) [4], and showed an *N*-methyl (δ_H 3.70) and two methoxyl groups at δ_H 3.93 and 4.21 ppm. The aromatic region of the spectrum was well resolved and exhibited the presence of an *ortho*-disubstituted benzene ring (Table 2). On the basis of these spectral data, structure (**9**) was assigned to *N*-methylswietenidine-B. The proposed structure was confirmed by the ^{13}C NMR spectrum (Table 2) which was fully assigned.

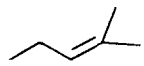
Except for *N*-benzoyl-2-hydroxy-2-(4-methoxyphenyl) ethylamine (**11**) [17] isolated from *C. brevistyla* Oliver and lansamide I (**12**) [18] clausenamide (**13**) [19], cycloclausenamide and neoclausenamide [20] from *C. lansium*, most alkaloids of the genus *Clausena* are carbazole derivatives probably derived from 3-methylcarbazole (**8**) [12, 21]. This is the first report of a 2-quinolone alkaloid from this taxon. As mentioned earlier, the only alkaloids previously isolated from this species include mupamine (**1**) [5, 6], clausanitin (**2**) [7] and atanisatin (**3**) [7]. In our own studies, none of these alkaloids was detected. It would therefore appear that the alkaloidal constituents of *C. anisata* vary with geographical location.

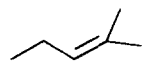


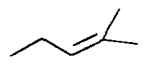


1 R = OMe
7 R = H

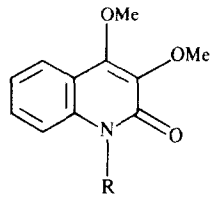
2 $R^1 = R^3 = R^5 = H$ $R^2 = OH$ $R^4 =$ 

3 $R^1 = R^3 = R^4 = H$ $R^2 = OMe$ $R^5 =$ 

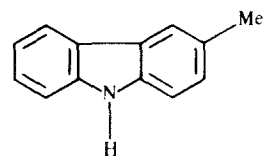
4 $R^3 = R^4 = R^5 = H$ $R^2 = OH$ $R^1 =$ 

5 $R^2 = R^4 = R^5 = H$ $R^1 = OMe$ $R^3 =$ 

6 $R^2 = R^3 = R^4 = R^5 = H$ $R^1 = OH$



9 R = Me
10 R = H



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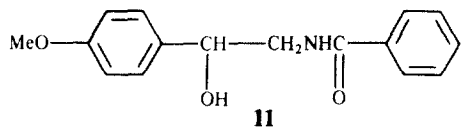

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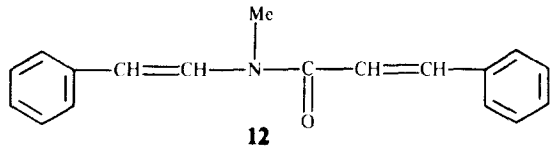

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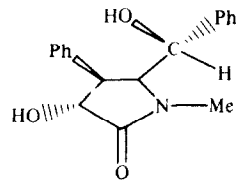

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EXPERIMENTAL

For general details consult ref. [8].

Extraction and isolation. The combined powdered stem bark and roots of *C. anisata* (8 kg) collected from Oku near Bamenda North-West Province of Cameroon, were extracted and chromatographed as previously reported [8]. The present study concerns the six uncharacterized alkaloids reported in a previous study [8]. The alkaloids purified by flash CC and prep. TLC are presented below.

Heptaphylline (**4**). Yellow needle crystals (hexane–EtOAc) (80 mg) mp 189–190° (lit. 190–191° [9, 10]).

Ekeberginine (**5**). Colourless plates ($CHCl_3$) (65 mg) mp 231–232° (lit. 230–231° [10]); IR ν_{max}^{KBr} cm^{-1}: 3460, 3160, 1645,

Table 1. NMR spectral data of *O*-demethylmurrayanine (**6**) (in CD_3OD at 25.02 MHz for ^{13}C and at 200.13 MHz for ^1H)

	^{13}C NMR*	^1H NMR
C-1	146.0 *s*	—
C-1a	131.3 *s*	—
C-2/H-2	108.4 *d*	7.33 *d* (1.4)†
C-3	118.0 *s*	—
C-4/H-4	127.3 *d*	8.15 *d* (1.4)
C-4a	109.5 *s*	—
C-5/H-5	121.0 *d*	8.08 *ddd* (7.8, 1.2, 0.8)
C-5a	116.0 *s*	—
C-6/H-6	120.1 *d*	7.21 *ddd* (7.8, 7.0, 1.2)
C-7/H-7	126.0 *d*	7.41 *ddd* (8.2, 7.0, 1.2)
C-8/H-8	112.7 *d*	7.53 *ddd* (8.2, 1.2, 0.8)
C-8a	145.2 *s*	
CHO	194.1 *d*	9.89 *s*

*Multiplicities were obtained by off-resonance decoupling experiments.

†Coupling constants (*J* in parentheses) are given in Hz.

Table 2. NMR spectral data of *N*-methylswietenidine-B (**9**) at 50.32 MHz for ^{13}C (shifts relative to $CDCl_3$ at δ_C 77.0) and at 200.13 MHz for 1H (shifts relative to $CDCl_3$ at δ_H 7.25)

	^{13}C NMR†	^1H NMR
C-2	161.2 *s*	—
C-3	136.6 *s*	—
C-4	151.5 *s*	—
C-5/H-5	123.6 *d*	7.93 *ddd* (8.0, 1.6, 0.5)*
C-5a	118.0 *s*	—
C-6/H-6	122.1 *d*	7.23 *ddd* (8.0, 7.1, 1.1)
C-7/H-7	129.6 *d*	7.50 *ddd* (8.5, 7.1, 1.6)
C-8/H-8	113.7 *d*	7.30 *ddd* (8.5, 1.1, 0.5)
C-8a	135.2 *s*	—
N-Me	29.5 *q*	3.70 *s*
3-OMe	60.5 *q*	3.93 *s*
4-OMe	60.8 *q*	4.21 *s*

*Coupling constants (*J* in parentheses) are given in Hz.

†Multiplicities of carbons were assigned from DEPT experiments.

1610, 1580, 1500, 1450, 1335, 1305, 1120, 850, 730; EIMS *m/z* (rel. int.): 293 $[M]^+$ (100), 278 $[M-Me]^+$ (37), 250 $[278-CO]^+$ (44).

O-*Demethylmurrayanine* (3-*formyl*-1-*hydroxylcarbazole*) (**6**). Colourless plates ($CHCl_3$) (40 mg) mp 237°–239°; IR ν_{max}^{KBr} cm^{-1}: 3460, 1670, 1610, 1580, 1500, 1490, 1470, 1450, 1420, 1375, 1350, 1315, 1250, 1160, 1120, 1100, 1000, 850, 725. UV λ_{max}^{EtOH} nm (log ε): 346 (4.22), 336 (4.22), 291 (4.45), 278 (4.59), 255 (4.39), 244 (4.51), 226 (4.40); for ^1H and ^{13}C NMR see Table 1. EIMS *m/z* (rel. int.): 211 $[M]^+$ (100), 210 $[M-H]^+$ (74), 183 (12), 182 (39), 155 (11), 154 (45), 128 (17), 127 (27), 126 (19), 77 (28); (found C, 73.90; H, 4.15; N, 6.55; $[M]^+$ 211.2065 $C_{13}H_9NO_2$ requires, C, 73.92; H, 4.29; N, 6.63%; $[M]^+$ 211.2091).

Girinimbine (**7**). Yellow crystals (45 mg) (petrol – EtOAc) mp 170–171° (lit. 174° [11]); UV λ_{max}^{EtOH} nm (log ε): 350 (3.90), 330 (4.00), 315 (3.98), 277 (4.45), 236 (4.70).

3-*Methylcarbazole* (**8**). Colourless crystals (30 mg) (petrol–EtOAc) 174–175° (lit. 176° [13]); EIMS *m/z* (rel. int.) 181 $[M]^+$ (100), 180 $[M-H]^+$ (94), 152 (17), 77 (15).

N-*Methylswietenidine-B* (1-*methyl*-3,4-*dimethoxy*-2-*quinolone*) (**9**). Colourless plates (CH_2Cl_2) (20 mg) mp 74–75°; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1630, 1600, 1495, 1460, 1355, 1315, 1280, 1220, 1145, 1100, 1040, 970; UV λ_{max}^{EtOH} nm (log ε): 330 sh (3.70), 318 (3.78), 286 (3.77), 231 (4.50); $\lambda_{max}^{EtOH+2M\ HCl}$ nm (log ε): no change; for ^1H and ^{13}C NMR see Table 2; EIMS *m/z* (rel. int.): 219 $[M]^+$ (67), 204 $[M-Me]^+$ (100), 188 $[M-OMe]^+$ (16), 176 $[204-CO]^+$ (24), 133 (13), 105 (13), 104 (14); (Found: C, 65.72; H, 5.80; N, 6.30; $[M]^+$ 219.2295 $C_{12}H_{13}NO_3$ requires: C, 65.74; H, 5.98; N, 6.39%, $[M]^+$ 219·2272.

Acknowledgements—The authors thank Mr Benoit MPOM (National Herbarium Yaounde, Cameroon) for the collection of plant material. One of us (B. T. Ngadjui) is grateful to the International Foundation for Science (Stockholm) for a grant F/933.

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