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FURTHER ALKALOIDS OF *ARALIOPSIS TABOUENSIS*: THE STRUCTURE OF ARALIOPSININE AND THE PRESENCE OF DIMERIC 2-QUINOLINE ALKALOIDS

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ABSTRACT. Fifteen quinoline alkaloids have been isolated from the stem bark of *Araloiopsis tabouensis*. One of them, araliopsinine, is new, while the other fourteen are known. Maculine, kokusaginine, flindersiamine, skimmianine, (+)-isoplatydesmine (-)-ribalinine, and N-methylplatydesminium cation have been previously isolated from this genus whereas veprisine, N-methylpreskimmianine, verpridimerinesA-D and arborinine are reported for the first time. In addition, the protolimonoid flindissol, the coumarin scoparone, and the triterpene lupeol, have also been identified. The implication of these results on previous investigations on this taxon are discussed.

INTRODUCTION

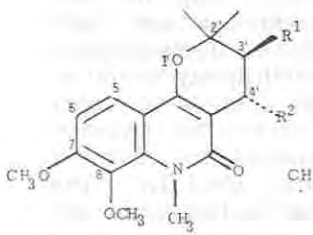
Araliopsis tabouensis Aubrev. & Pellegr. (Rutaceae) is a large Tropical West African tree whose bark used in folk medicine (1) for the treatment of gonorrhoea. In previous investigations of this species (2,3), large quantities of the protolimonoid, flindissol, and several quinoline alkaloids were identified. The sister species, *A. soyauxii* Engl. (4), now considered to be conspecific with *A. tabouensis* (Waterman, P.G. personal communication) however yielded a different alkaliod spectrum (5). Recently, we found that the bark of another Taddalioideae, *Vepris louisii*, contained both monomeric and unusual dimeric 2-quinolone alkaloids (6,7). In view of these findings and the close morphological relationship between *V. louisii* and *A. tabouensis* on the one hand (4), and the apparently conflicting reports on the chemistry of the latter plant (2,3,5) on the other hand, we were interested in identifying the chemical constituents of *A. tabouensis* occurring in Cameroon. We now report the results of an examination of the bark (root and trunk) of this tree.

In addition to the compounds previously reported from this taxon viz: N-methylplatydesminium cation (3), skimmianine (3,5), (+)-isoplatydesmine (3,5), (-)-ribalinine (5), flindersiamine (5), maculine (5), kokusaginine (5), and flindissol, the known compounds scoparone, lupeol, arborinine (8), veprisine (9), N-methylpreskimmianine (9), and the dimeric 2-quinolones, vepridimerines A-D (7) were also isolated. Only one new alkaloid, for which we have proposed the trivial name araloipsinine, was obtained. Whereas the other alkaloids are common rutaceous constituents, this is the second report of the unusual heptacyclic bis-2-quinolone alkaloids, vepridimerines A-D, from this family.

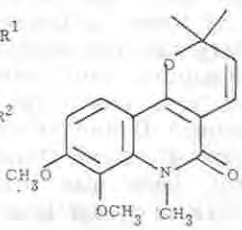
RESULTS AND DISCUSSION

The combined hexane and chloroform extracts, after precipitaion of flindissol, was subjected to column chromatography over silica gel and eighteen compounds (fifteen alkaloids and three neutral products) were either eluted singly or as mixures that were subsequently purified by repeated column chromatography or preparative TLC. The known compounds were identified by comparison with authentic samples available from previous studies in our laboratory, except scoparone which was prepared from scopoletin by methylation with diazomethane.

Araliopsinine (**1**). Colourless needles, $C_{17}H_{21}NO_6$, mp 186-187° $[\alpha]^{24}$ +2.6° ($CHCl_3$; c 1.01), showed UV λ_{max} (EtOH): 312 (logε 4.18), 293 (4.23), 254 (4.65), 247 (4.51) and 237 nm (4.30). It exhibited a very simple ^1H NMR spectrum which indicated two *ortho*-coupled aromatic protons (δ_H 7.70, (1H,d, J = 9 Hz) and 6.87 (1H, d, J = 9 Hz), two methoxy groups at δ_H 3.95 and 3.92 (each 3H,s), an



1 $R^1 = R^2 = OH$
1a $R^1 = R^2 = OAc$
1b $R^1 = OH$ $R^2 = OAc$



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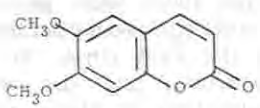

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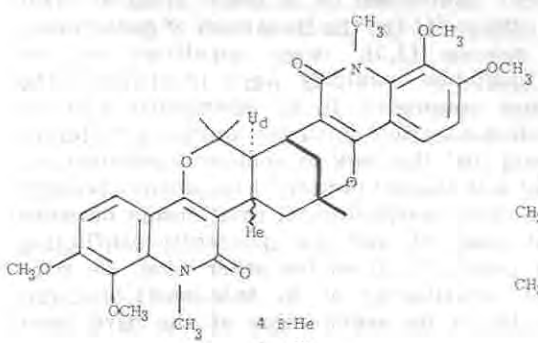
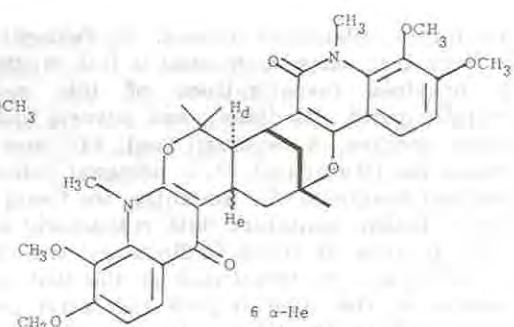


4 β-He
5 α-He



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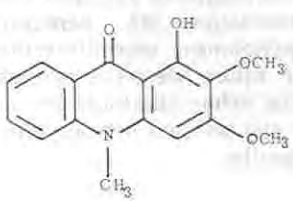
7 β-He



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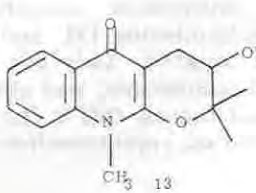
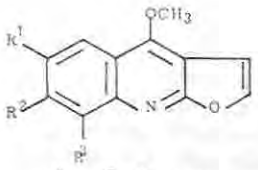


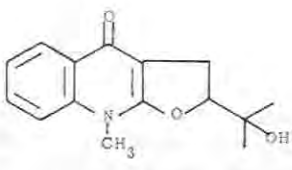
13

N-methyl δ3.78 (3H, s), and a gem-dimethyl group δ_H 1.58 and 1.27 (each 3H, s). In addition, two *vicinal* protons occurring as two one-proton doublets at δ 4.72 and 3.85 (J = 8 Hz) were also clearly discernable. The above spectral data suggest that araliopsinine is either the dihydroxylated derivative (**1**) of veprisine (**4**) or the isomeric biogenetically interesting (10) 4-quinolone (**2a**).

The latter structure was however eliminated on the following grounds: Mild acetylation of araliopsinine (**1**) with cold acetic anhydride in pyridine (1:1) readily afforded a diacetate (**1a**) rather than a monoacetate thus ruling out the presence of a tertiary hydroxyl group in **1**, which would have not undergone easy acetylation (cf the conditions of the diacetylation of myrtopsine (11)). Furthermore, in the ^{13}C NMR of the diacetate (**1a**), the carbonyl resonance was observed at δ_C 163.5 indicating the presence of a 2-quinolone (7) rather than of a 4-quinolone which otherwise would have absorbed at δ_C ca 171–175 ppm (7). Structure **1** (3',4'dihydroxy-3',4'-dihydroveprisine) was thus proposed for araliopsinine. The magnitude of the coupling constant $J_{3',4'}$ 8 Hz in the ^1H NMR spectrum of (**1**) showed a *trans* configuration for araliopsinine and further suggested a diequatorial disposition for the 3'- and 4'-hydroxyl groups (12,13). In the ^1H NMR spectrum of the diacetate (**1a**) however, $J_{3',4'}$ is diminished to 3 Hz presumably due to conformational change to the *trans*-diaxial arrangement inorder to minimize unfavourable interactions between the neighbouring more bulky acetoxy groups (3). The structure of araliopsinine was finally confirmed as *trans* 3',4'-dihydroxy3',4'-dihydroveprisine (**1**) by its synthesis in the racemic form from veprisine (**2**) on treatment with *meta*-chloroperbenzoic acid. The spatial disposition of the hydroxyl groups in the 3'- and 4'-positions was also determined as in **1a-1b** by further chemical correlation with veprisine (**2**). Oxidation of veprisine (**2**) in acetic acid with chromic oxide (14,15) afforded the (+_)-trans-4'-acetoxy-3',4'dihydroveprisine (**1b**). Direct hydrolysis of the crude reaction mixture with sodium methoxide gave racemic (**1**) identical in all respects (TLC,UV, IR, ^1H NMR) with the natural sample.



9 R^1=H, R^2= R^3 = OCH_3
10 $R^1 + R^2$ = $-OCH_2O-$, R^3 = H
11 $R^1 + R^2$ = $-OCH_2O-$, R^3 = OCH_3
12 R^1 = R^2 = OCH_3, R^3 = H



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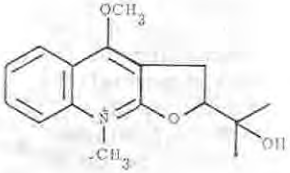

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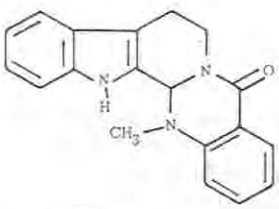

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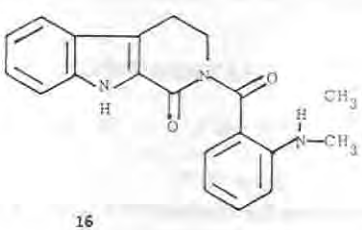

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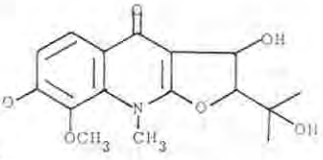

2a

A comparative study of all the secondary metabolites obtained so far from the bark of *A. tabouensis* in the present investigation and in two previous studies (2,3,5) is presented in Table 1. Major differences are apparent from the spectrum of compounds reported in the three studies.

The most striking difference is the occurrence of monomeric as well as the dimeric 2-quinolone alkaloids. In the present study, a total of seven members of this group of alkaloids including three as yet uncharacterised members of this group of alkaloids was obtained from *A. tabouensis*. In none of the other

Table 1 Constituents of *A. tabouensis* from three separate investigations of barks collected from three different geographical locations.

Sample	*A. tabouensis* Root and stem bark	*A. soyauxii*[1] Root and stem bark	*A. tabouensis* Root and stem bark
Origin	Ghana	Gabon-Cameroon Frontier	Cameroon
Reference	Fish and co-workers (2,3)	Vaquette et. al. (5)	Present study
Compound	Isolation	Isolation	Isolation
Flindissol	+	-	+
Lupeol	-	-	+
Scoparone (3)	-	-	+
N-Methylpreskimmianine	-	-	+
Veprisine (2)	-	-	+
Araliopsinine (1)	-	-	+
VepridimerineA (5)	-	-	+
VepridimerineB (4)	-	-	+
VepridimerineC (6)	-	-	+
VepridimerineD (7)	-	-	+
Arborinine (8)	-	-	+
N-Methylplatydesminium cation (15)	+	-	+
(+)-Isoplatydesmine (14)	+	+	+
(+)-Ribalinine (13)	+	-	-
(-)-Ribalinine (13)	-	+	+
Araliopsine	-	+	-
Evodiamine (17)	+	-	-
Rhetsinine (16)	+	-	-
Skimmianine (9)	+	+	+
Kokusaginine (12)	-	+	+
Maculine (10)	-	+	+
Flindersiamine (11)	-	+	+
Halfordinine	+	-	-
Other unidentified compounds	+	-	+

A. soyauxii is now considered to be conspecific with *A. tabouensis* (Waterman, P.G., Private communication)

\+ : Compound isolated by these authors

\- : Compound not isolated by these authors.

three publications has there been a report of these alkaloids. This may be due to one or a combination of a number of reasons. 2-Quinolones (monomeric or dimeric), being lactams are very weak bases and do not readily form hydrochlorides. Vaquette and coworkers (5) worked only on the basic tertiary alkaloids isolated via their hydrochlorides. In addition, the petroleum ether extract that normally contains veprisine (**2**) and N-methylpreskimmianine was not examined by these authors. It is therefore understandable that they did not report these compounds. It is however not immediately clear why Fish *et al* (3) failed to report the isolation of these alkaloids from the Ghanaian variety of *A. tabouensis* (studied as *A.soyauxii*). But if this variety contains these alkaloids in the relatively low concentrations encountered in the Cameroonian material, then the small quantity of the bark used (1 kg) and difficulties in isolation and purification may simply have precluded their detection and identification. Secondly, Fish and collaborators (3) tentatively identified a mixture of rhetsinine (**16**) and evodiamine (**17**) in the original chloroform extract from which tertiary alkaloids had been removed by treatment with hydrochloric acid (1N). Paucity of material however precluded their resolution into and positive identification as the two indolopyridoquinazolines. Vaquette et al (5), on the other hand did not isolate these alkaloids from the Gabonese specimen, and though we have obtained indolopyridoquinazoline derivatives from the related Toddalioideae, *Vepris lousisii* (6), we were unable to identify rhetsinine or evodiamine even in trace amounts in our sample. It is possible that the Ghanaian variety of *A. tabouensis* could contain these two alkaloids while the Cameroonian and Gabonese materials are devoid of them (6). But we consider this particular report (3) in error for the following reason. Rhetsinine (**16**) and evodiamine (**17**) contain at least one secondary and one tertiary nitrogen atoms and readily form hydrochlorides with dilute HCl solutions (17,18,19). There is no reason why the chloroform extract after treatment with HCl (1N) should still contain (**16**) and (**17**). In an attempt to isolate these compounds, we treated the chloroform extract following the method of Fish et al (3) and also obtained a yellow precipitate which gave basically the same mass spectrometric fragments as those recorded by Fish and co-workers. The precipitate however turned out, on chromatographic purification, to be a mixture of arborinine (**8**) (8) and vepridimerines-A,B,C and D. Arborinine does not normally form a hydrochloride (20).

Finally, a noteworthy variation is evident in the occurrence of anthranilic acid derived alkaloids in *A. tabouensis* collected from the three different localities. Maculine (**9**), flindersiamine (**10**), kokusaginine (**12**) and (-)-ribalinine (**13**) in the Gabonese (5) and Cameroonian populations are replaced by (+)-ribalinine and halfordinine in the Ghanaian variety (3). This variation of chemical constituents of plant species with geographical location of the species is not without precedent among West African Toddaliodeae. Analogous situations have been reported with *Oricia sauveolens* (16) and *Teclea verdoorniana* (21). Waterman (16) has suggested that such diversity in alkaloids production by a given plant species in different environments might be a sign of adaptation to different factors in the various habitats.

EXPERIMENTAL

Melting points were determined on a Kofler hot plate and are uncorrected. UV spectra were recorded in EtOH solutions on a Beckmann 25 instrument and IR spectra (KBr-discs) on a PerkinElmer 727 B spectrophotometer. Mass spectral analyses were carried out with an LKB 9000 machine at 70 eV with direct inlet. NMR spectra were recorded in deuterochloroform at 60 MHz (unless stated otherwise) with either a Perkin-Elmer R 12 A or R 12 B spectrometer. Chemical

shifts are in ppm downfield from tetramethylsilane as internal standard. Optical Rotations were determined on an AA-100 digital polarimeter.

Plant material. A. tabouensis, root and stem bark, was collected on the outskirts of Kumba, Cameroon, in February 1983 by Benoit Mpom and confirmed by the Cameroon National Herbarium, Yaounde, where a voucher specimen has been deposited.

Isolation of Compounds. The air-dried powdered bark (root and trunk) (5 kg) was successively extracted in a Soxhlet with n-hexane (20 l), chloroform (20 l), and methanol (20 l). The hexane extract on standing deposited large amounts of a white solid which on filtration and recrystallisation from ether afforded flindissol (82 g) (1). The resulting filtrate and the chloroform extract were found to be similar on TLC analysis (C_6H_6-EtOAc, 4:1 and 3:2) and were thus combined. Part (120 g) of the total extract (604 g) was chromatographed over silica gel 1500 g) packed in hexane. Gradient elution was effected with hexane, hexane-ether mixtures, chloroform and chloroform-methanol mixtures. A total of 322 fractions of about 250 ml each were collected and combined on the basis of TLC and ^1H NMR data . The pure compounds were obtained from the combined fractions either by direct crystallisation or after further purification by CC or preparative TLC. In this way, the following compounds described in order of their elution from the column were characterized: lupeol (28 g), N-methylpreskimmianine (350 mg), veprisine (**2**), (4 g), scoparone (**3**), (85 mg), flindissol (26 g), vepridimerine-B (**4**) (68 mg), vepridimerine-A (**5**) (28 mg), arborinine (**8**) (18 mg), araliopsinine (**1**) (98 mg), skimmianine (**9**) (28 mg), maculine (**10**) (32 mg) flindersiamine (**11**) (42 mg), kokusaginine (**12**) (40 mg), vepridimerine-C (**6**) (48 mg), vepridimerine-D (**7**) (28 mg), (-)-ribalinine (**13**) (1.2 g), and (+)isoplatydesmine (**14**) (2.8 g). Furthermore, part (100 g) of the methanol extract was washed with chloroform (to remove traces of tertiary alkaloids), acidified to pH 2 (5 % HCl), and treated with a solution of Mayer's Reagent (14). The precipitated, yellow quaternary alkaloidal complex was converted into the chlorides with Amberlite IRA 400 in the Cl^- form. Purification of the quaternary salts by silica gel coloumn chromatography (elution with chloroform-methanol, 1:1) afforded N-methylplatydesminium chloride (**15**) (6 g) as the major compound. Two other minor compounds revealed by TLC are under investigation. An unresolved mixture of dimeric 2-quinolone alkaloids apparently not closely related to the vepridimerines was also isolated from the chloroform extract. Known compounds were identified by direct comparison (mp, UV, IR and ^1H NMR) with authentic samples and will therefore not be described here.

Araliopsinine (1). Colourless needles (hexane-Et_2O), m.p. 186-187°; $[\alpha]^{24}$ = + 2.6° (c 1.01, $CHCl_3$); IR νmax cm^{-1}: 3350 (bonded OH), 1650 (2-quinolone C=O), 1580, 1470, 1420, 1390, 1350, 1330, 1300, 1280, 1230, 1200, 1140, 1080, 1060, 1030, 970, 910, and 840. ^1H NMR : δ 7.70 (1H, d, J = 9 Hz, H-5), 6.87 (1H, d,J = 9 Hz, H-6), 5.55 (1H, bs, exchanged with D_2O, OH), 4.72 (1H,d, J = 8 Hz, H4'), 3.95 (3H, s, OMe), 3.92 (3H, s OMe), 3.85 (1H, d, J = 8 Hz, H-3'), 3.78 (3H, s, N-Me), 3.15 (1H, bs, exchanged with D_2O, OH), 1.58 (3H, s, CH_3) and 1.27 (3H, s, CH_3). EIMS m/z (rel. int.) : 335 (M^+, 10%), 265(18), 264(100), 256(6), 238(4), 236(4), 235(13), 129(5), 73(12), 67(19), and 69(17). Found : C, 60.93; H, 6.40; N, 4.31. $C_{17}H_{21}O_6N$ requires : C,60.88; H, 60.31; N, 4.18%.

Acetylation of Araliopsinine (1). Araliopsinine (1) (50 mg) in dry pyridine (4 ml) was treated with Ac_2O (3 ml) at room temperature for 12 hr. The usual workup gave an oily residue (43 mg). Crystallization of the oil from Et_2O-CH_2Cl_2 afforded the diacetate (**1a**) (38 mg), colourless prisms: mp 208°; IR (KBr) ν_{max} cm^{-1}: 1760 (acetate C=0), 1650 (2-quinolone C=0), 1610. ^1H NMR (90 MHz): δ_H 7.7 (1H, d, J =9Hz, H-5), 6.85 (1H, d, J = 9 Hz, H-6), 5.91 (1H, d, J = 3Hz, H4'), 5.25 (1H, d, J = 3Hz, H-3'), 3.96 (3H, s, OMe), 3.90 (3H, s, OMe), 3.78 (3H, s, NMe), 2.05 (6H, s, 2 x COMe), 1.48 (3H, s, Me), and 1.4 (3H, s, Me). δ_C 169.7

(2) (acetates), 163.5 (C-2), 156.7, 156.1, 119.8 (C-5), 107.1 (C-6), 71.7 (C-4'), 64.7 (C-3'), 61.7 (8-OMe), 56.3 (7-OMe), 33.4 (N-Me), 23.8, 23.7, 20.9 and 20.8 (methyls). Found: C, 59.98; H, 6.12; N, 3.52. $C_{21}H_{25}O_8N$ requires: C, 60.13; H, 6.01; N, 3.34 %.

Conversion of Veprisine to (+)-Araliopsinine (1). A soln. of veprisine (2) (1.5 g) and m-CPBA (3 g) in $CHCl_3$ (25 ml) was stirred at room temp for 48 hr. The reaction mixture was washed with 5% aq. $NaHCO_3$, and the $CHCl_3$ evaporated. A soln. of the resulting residue (1.3 g; 2 major products as shown by TLC) in H_2SO_4 (40%, 40 ml) was refluxed for 30 min. after which time it was cooled, diluted with H_2O (100 ml), and extracted with $CHCl_3$ (3 x 30 ml). The combined $CHCl_3$ extracts were washed (H_2O), dried (Na_2SO_4) and evaporated to yield a gum (500 mg). Purification of the gum (silica gel CC, EtOAc : MeOH 99:1) yielded colourless plates (350 mg), mp 185-196° identical in all respects (IR, UV, and EIMS) with the natural araliopsinine (1).

(+)-Trans-4'-acetoxy-3'-hydroxy-3',4'-dihydroveprisine and (+)-trans-3',4'-dihydroxy3',4'-dihydroveprisine (1) from CrO_3 oxidation of Veprisine (2).

A solution of veprisine (2) (300 mg) in AcOH (12 ml) was added to a solution of CrO_3 (100 mg) in AcOH (5 ml) and the resulting mixture stirred at 40° for 2 hr. The reaction mixture was poured into H_2O (75 ml) and extracted with $CHCl_3$ (3 x 50 ml), dried with anhydrous Na_2SO_4 and evaporated *in vacuo*. The semisolid obtained (265 mg) showed one major spot and three minor ones on TLC. A solution of the residue (250 mg) in MeONa (0.3N, 10 ml) in MeOH was then stirred at room temperature for 4 hr, neutralized with Amberlite IR C 50 in the H^+ form and evaporated under reduced pressure. The residue obtained (220 mg) was chromatographed on a short silica gel column to give (+)-3',4'-dihydroxy-3',4'-dihydroveprisine (1) identical (TLC, UV, IR, MS, and ^1H NMR) with the natural compound.

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REFERENCES

1. F.R. Irvine in "Woody Plants of Ghana", p.491, Oxford University Press, (1961).
2. P.G. Waterman, Biochem. Syst. Ecol., **1**, 153 (1973).
3. F. Fish, A.I. Meshal and P.G. Waterman, Planta Med., **29**, 310 (1976).
4. R. Letouzey, Flore du Cameroun, Vol. 1, Museum National d'Hisoire Naturelle, Paris, 99 (1963).
5. J. Vaquette, M.S. Hifnawy, J.L. Pousset, A. Fournet, A. Bouquet and A. Cave, Phytochemistry, **15**, 743 (1976).
6. J.F. Ayafor, B.L. Sondengam and B.T. Ngadjui, Phytochemistry, **21**, 2733 (1982).
7. B.T. Ngadjui, J.F. Ayafor, B.L. Sondengam, J.D. Connolly, D.S. Rycroft, S.A. Khalid, P.G. Waterman, N.M.D. Brown, M.F. Grundon, and V.N. Ramachandran, Tetrahedron Letters, **23**, 2041 (1982).
8. S.C. Pakrashi, S.K. Roy, L.F. Johnson, T. Georges and C. Djerassi, Chem. Ind., 464 (1961).
9. J.F. Ayafor, B.L. Sondengam and B.T. Ngadjui, Tetrahedron Letters, **21**, 3293 (1980).

10. M.F. Grundon, D.M. Harrison and G.G. Spyropoulos, J. Chem. Soc. Chem. Commun., 51 (1974).
11. M.S. Hifnawy, J. Vaquette, T. Sevenet and J.L. Pousset, A. Cave, Planta Med., **29**, 346 (1976).
12. W.D. Cotterill, J. Cottam and R. Livingston, J. Chem. Soc. (C), 1006 (1970).
13. R.C. Jennings and A.P. Ottridge, J. Chem. Soc. Perkin Trans. I , 1733 (1984).
14. S.Mitaku, A-L. Skaltsounis, F. Tillequin and M. Koch, J. Nat. Prod., **48**, 772 (1985).
15. R.C.F. Brown, J.J. Hobbs, G.K. Hughes and E. Ritchie, Aust. J. Chem., **7**, 348 (1954).
16. P.G. Waterman, A.I. Meshal, J.B. Hall and M.D. Swaine, Biochem. Syst. Ecol., **6**, 239 (1978).
17. T. Kamikado, S. Murakoshi and S. Tamura, Agric. Biol. Chem., **42**, 1515 (1978).
18. C.L. King, Y.C. Kong, N.S. Wong, H.W. Yeung, H.H.S. Fong, U. Sankawa and J. Nat. Prod., **43**, 577 (1980).
19. B. Danielli, P. Manitto, F. Ronchetti and G. Russo, Phytochemistry, **11**, 1833 (1972).
20. K.H. Pegel and W.G. Wright, J. Chem. Soc. (C), 2327 (1969).
21. J.F. Ayafor and J.I. Okogun, J. Chem. Soc., Perkins Trans. I, 909 (1982).