Phytochemistry, Vol. 25, No. 7, pp. 1691–1695, 1986.
Printed in Great Britain.

0031-9422/86 $3.00 + 0.00
Pergamon Journals Ltd.

AZAFLUORENONES AND AZAANTHRAQUINONE FROM *GUATTERIA DIELSIANA**

MARÍLIA O. F. GOULART, ANTÔNIO EUZÉBIO G. SANT'ANA, ALAÍDE B. DE OLIVEIRA†, GEOVANE G. DE OLIVEIRA† and JOSÉ GUILHERME S. MAIA‡

Departamento de Química, CCEN, Universidade Federal de Alagoas, 57.000, Maceió, Brazil; †Departamento de Química, ICEx, Universidade Federal de Minas Gerais, 30.000, Belo Horizonte, Brazil; ‡Departamento de Produtos Naturais, INPA, 69.000, Manaus, Brazil

(*Received* 15 *October* 1985)

Key Word Index—*Guatteria dielsiana*; Annonaceae; trunkwood; alkaloids; oxoaporphines; azafluorenones; azaanthraquinone; triterpene; polycarpol; structural elucidation; biosynthetic proposals.

Abstract—Trunkwood of *Guatteria dielsiana* afforded eight alkaloids and one triterpene. Three of the alkaloidal constituents are oxoaporphines: liriodenine, *O*-methylmoschatoline and isomoschatoline; four are 1-azafluorenones: onychine, the only previously known representative of this group, and three novel compounds of this rare type, 6-methoxyonychine, dielsine (1-aza-4-methyl-2-oxo-1,2-dihydrofluorenone) and dielsinol (1-aza-4-hydroxymethyl-2-oxo-1,2-dihydrofluorenone). The last alkaloid is dielsiquinone (1-aza-3-methoxy-4-methyl-2-oxo-1,2-dihydro-9,10-anthracenedione), the second representative of a new class of quinones of which cleistopholine was the only one previously known. The triterpene is polycarpol whose presence is of chemotaxonomic significance since this metabolite seems to be exclusive to the Annonaceae.

INTRODUCTION

The Annonaceae is a large family comprising *ca* 130 genera and 2300 species. Phytogeographically it is entirely tropical, 39 genera being represented in America [1]. The Annonaceae form part of the Ranalean alliance [2], a group recognized as being the centre for benzylisoquinoline alkaloid production in plants [3]. Indeed the great majority of alkaloidal constituents of these plants are isoquinoline-derived structures, along with a wide range of non-alkaloidal compounds of varied structural types [4, 5]. African species of Annonaceae have led to novel and unusual compounds derived from different biogenetic pathways [6, 7]. The present paper describes the chemical investigation of the trunkwood from *Guatteria dielsiana* collected in the vicinity of Manaus, Amazonas State, and identified by Dr. W. A. Rodrigues (INPA, Manaus), voucher 2861, INPA, Manaus.

RESULTS AND DISCUSSION

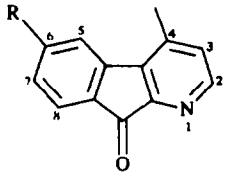
Chromatographic fractionation of the chloroform-soluble bases of the trunkwood extractive from *G. dielsiana* over neutral alumina afforded two closely related alkaloids (**1** and **2**). The chemical similarity of these compounds was indicated by the IR and UV spectra which showed for both of them an aromatic character and the presence of a conjugated carbonyl group. Mass measurements indicated the $[M]^+$ peaks at *m/z* 195 and 225 that can be related to the empirical formulae $C_{13}H_9NO$ and $C_{14}H_{11}NO_2$. The ^1H NMR spectra revealed the presence of a γ-methylpyridine unit in both of them, an *ortho*-disubstituted benzene ring for **1**, while for **2**, a 1,2,4-trisubstituted benzene ring, bearing a methoxyl group, was evident (Table 1). These data led to the identification of the first compound as onychine (**1**) and to the assignment of the structure of 6-methoxyonychine (**2**) to the second alkaloid. Allocation of the methoxyl group to C-6 (**2**) instead of C-7 was considered reasonable on the basis of the shielding effects experienced by the benzene protons as compared with those in **1** (Δδ: H-5 0.32; H-7 0.52; H-8 0.19). The enhanced Δδ value for H-8, which corresponds to approximately double that usually found for the diamagnetic effect of a methoxyl on a *meta* benzene proton, was taken as a consequence of a decrease in the anisotropy of the carbonyl due to its conjugation with the methoxyl group.

Onychine (**1**) was first isolated from the trunkwood of a Brazilian Annonaceae, *Onychopetalum amazonicum* [8], and it was recently found in the root of an African Annonaceae, *Cleistopholis patens* [6, 7]; **2** is a novel compound.

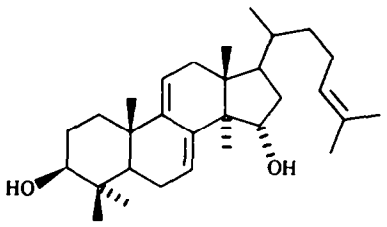
The HCl-insoluble chloroform extract afforded seven compounds (see Experimental): one triterpene and six alkaloids. Full spectral analysis of the triterpene and its products of acetylation and CrO_3 oxidation led to its characterization as polycarpol (**3**) [9]. Direct comparison with an authentic sample confirmed the identification of this rare triterpene.

Three of the alkaloidal constituents were characterized as oxoaporphines and they were identified by comparison with reported data as the widespread liriodenine, *O*-methylmoschatoline [10] and isomoschatoline, the latter described only for *Guatteria melosma* and *Cleistopholis patens* [11].

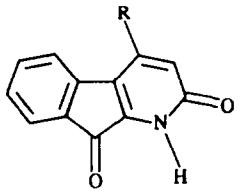
* Part of the Doctorate thesis submitted by M. O. F. Goulart at Universidade Federal de Minas Gerais. This work was first presented at the 14th International Symposium on the Chemistry of Natural Products, Poznán, 9–14 July 1984.



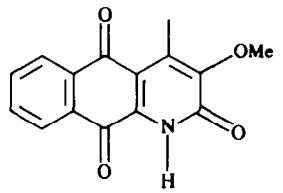
1 R = H
2 R = OMe



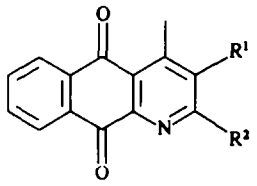
3



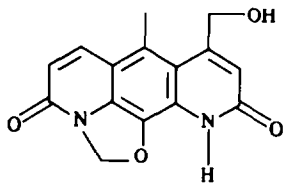
4 R = Me
5 R = CH_2OH



6



7 $R^1 = R^2 = H$
8 R^1 = OMe; R^2 = OH



12

Table 1. ^1H NMR chemical shift values (ppm) and coupling constants (Hz, in brackets) for alkaloids 1–6

Substances	H-2	H-3	H-5	H-6	H-7	H-8	Me-4	OMe	CH_2OH
Onychine*	8.42	6.98	7.72	7.60	7.44	7.86	2.64	—	—
(1)	*d*	*d*	*d*	*t*	*t*	*d*	*s*	—	—
	(5.6)	(5.6)	(7.6)	(7.6)	(7.6)	(7.6)			
6-Methoxy-	8.44	7.00	7.40		6.92	7.67	2.64	3.96	
onychine*	*d*	*d*	*d*	—	*dd*	*d*	*s*	*s*	—
(2)	(5.6)	(5.6)	(2.5)		(8.6)	(8.6)			
					(2.5)				
Dielsine†		7.18		7.7–7.9 *m* (2H)			2.42		
(4)	—	*br s*		8.0–8.3 *m* (2H)			*s*	—	—
Dielsinol†		7.32		7.7–7.9 *m* (2H)					4.83, *d*
(5)									(5.0) (2H)
	—	*br s*		8.0–8.2 *m* (2H)			—	—	4.2–4.4, *br t*
									(5.0) (1H)
Dielsiquinone‡				7.5–7.8 *m* (2H)			2.63	4.17	
(6)	—	—		8.0–8.3 *m* (2H)			*s*	*s*	—

*400 MHz, $CDCl_3$.
†100 MHz, Me_2CO-d_6.
‡60 MHz, $CDCl_3$–CD_3OD.

The other three alkaloids are new and their structures were established by spectral analysis. Accurate mass measurements led to the empirical formulae $C_{13}H_9NO_2$ and $C_{13}H_9NO_3$ for two of them, dielsine (4) and dielsinol (5). In comparison with onychine (1), they possess one and two additional oxygen atoms, respectively, if a common nucleus of a 1-aza-4-methylfluorenone is supposed. However, differences in the spectral behaviour of these two compounds in relation to those previously discussed (1 and 2) were noticed. Their IR spectra indicated the

presence of a lactam group [NHCO free and dimeric 3420, 3190 cm^{-1} (**4**); 3400, 3250 cm^{-1} (**5**); ν_{CO} 1665, 1650 cm^{-1} (**4**), 1655 cm^{-1} (**5**)] and their UV spectra did not change upon addition of HCl to the neutral ethanolic solution, but showed bathochromic shifts in the presence of conc. sodium hydroxide (2.5 N). This behaviour is consistent with a pyridone derivative and indeed the ^1H NMR spectra showed a one proton singlet at δ7.18 (**4**) and 7.32 (**5**) supporting the location of an oxygen function at C-2. The methyl group (δ2.42) in **4** was replaced by a hydroxymethyl group (δ4.30, $CH_2\underline{O}H$; 4.83, $C\underline{H}_2OH$) in **5** (Table 1). The discussed data led to the structures of 1-aza-4-methyl-2-oxo-1,2-dihydrofluorenone for dielsine (**4**) and 1-aza-4-hydroxymethyl-2-oxo-1,2-dihydrofluorenone for dielsinol (**5**).

The last compound, dielsiquinone (**6**), gave an $[M]^+$ peak at *m/z* 269 (100%) and fragment ion peaks at *m/z* 268 $[M-1]^+$ (30%), 254 $[M-Me]^+$ (15%), 241 $[M-CO]^+$ (6%), 240 $[M-CO-H]^+$ (16%) and 212 $[M-H-2\times CO]^+$ (6%). The difference in mass units (58 mu) between dielsine (**4**) and dielsiquinone (**6**), besides the fragmentation pattern, could be attributed to the presence of a methoxyl group and an additional CO group, suggesting a quinonoid structure closely related to cleistopholine (**7**), a metabolite from *Cleistopholis patens* [6, 7]. Indeed, the presence of a three-proton singlet at δ4.17 and the absence of a signal for the deshielded olefinic proton which is observed at lowfield (δ7) in the spectra of compounds **4** and **5** (Table 1) indicated a completely substituted pyridone nucleus, leading to the structure of 1-aza-3-methoxy-4-methyl-2-oxo-1,2-dihydro-9,10-anthracenedione for dielsiquinone (**6**). It represents, together with cleistopholine (**7**) [7], the first representatives of a new class of azapolycyclic alkaloids. The enhanced acidity of dielsiquinone (**6**), revealed by the bathochromic shift of the UV spectra in the presence of sodium acetate, is explained by the conjugation of the hydroxyl group with one of the carbonyl groups in the tautomeric form (**8**) that must be present in the ethanolic solution.

The co-occurrence of 1-azafluorenones and 1-azaanthraquinones in *Gutteria dielsiana* and *Cleistopholis patens* [6, 7] suggests their biogenetic relationship. A shikimate-acetate pathway with a 2-aminonaphthoquinone (**9**) intermediate derived from shikimate (**10**) and glutamic acid (**11**) by analogy with the biosynthesis of anthraquinones [12] is proposed. Closure of the pyridone ring by incorporation of two acetate units results by analogy with the established route observed for nibomycin (**12**), a metabolite from *Streptomyces* sp. [13]. Loss of CO would convert a 1-azaanthraquinone into a 1-azafluorenone. This conversion is proposed also by Cavé *et al.* and Waterman, although these azapolycyclic compounds are postulated as deriving by degradation of oxoaporphines (**13**) [14]. In a previous proposal, onychine (**1**) [8] was considered as derived from phenylalanine and mevalonate precursors, a pathway that is not directly applicable to the azaanthraquinones, as pointed out previously [6] (Scheme 1).

EXPERIMENTAL

MPs are uncorr. ^1H NMR spectra were recorded at 60 MHz, 100 MHz, and 250 MHz and at 400 MHz on an experimental apparatus at the University of Paris-Sud (Orsay, France); TMS was used as int. std. TLC spots were developed by 2% ceric sulphate soln in H_2SO_4 and heating at 100°. UV: EtOH–H_2O (9:1); IR: KBr discs; EIMS: 70 eV.

Extraction and isolation. Dried ground trunkwood of *G. dielsiana* R. E. Fries (7 kg) was exhaustively extracted with EtOH and the extract evapd to dryness under red. pres. The residue was then extracted several times with $CHCl_3$, yielding a $CHCl_3$ extract (69.50 g) and an insoluble fraction (46.50 g). Part of the $CHCl_3$ extract (25.00 g) was shaken out with 1.6 N HCl (3 × 300 ml). The combined acid extracts were made alkaline with conc. NH_4OH and the alkaloids extracted into $CHCl_3$. The combined organic layers were dried (Na_2SO_4) and removal of the solvent gave 3.32 g of crude bases. The crude alkaloids (3.32 g) were chromatographed over alumina and eluted with C_6H_6, C_6H_6–$CHCl_3$, $CHCl_3$, $CHCl_3$–MeOH and finally MeOH. The resultant fractions were combined according to indications from TLC. One of the groups (eluted with C_6H_6–$CHCl_3$, 1:1) was rechromatographed over silica gel, eluted with mixtures of increasing polarity of Et_2O–EtOAc giving two compounds, onychine (**1**, 0.004 g) and 6-methoxyonychine (**2**, 0.005 g).

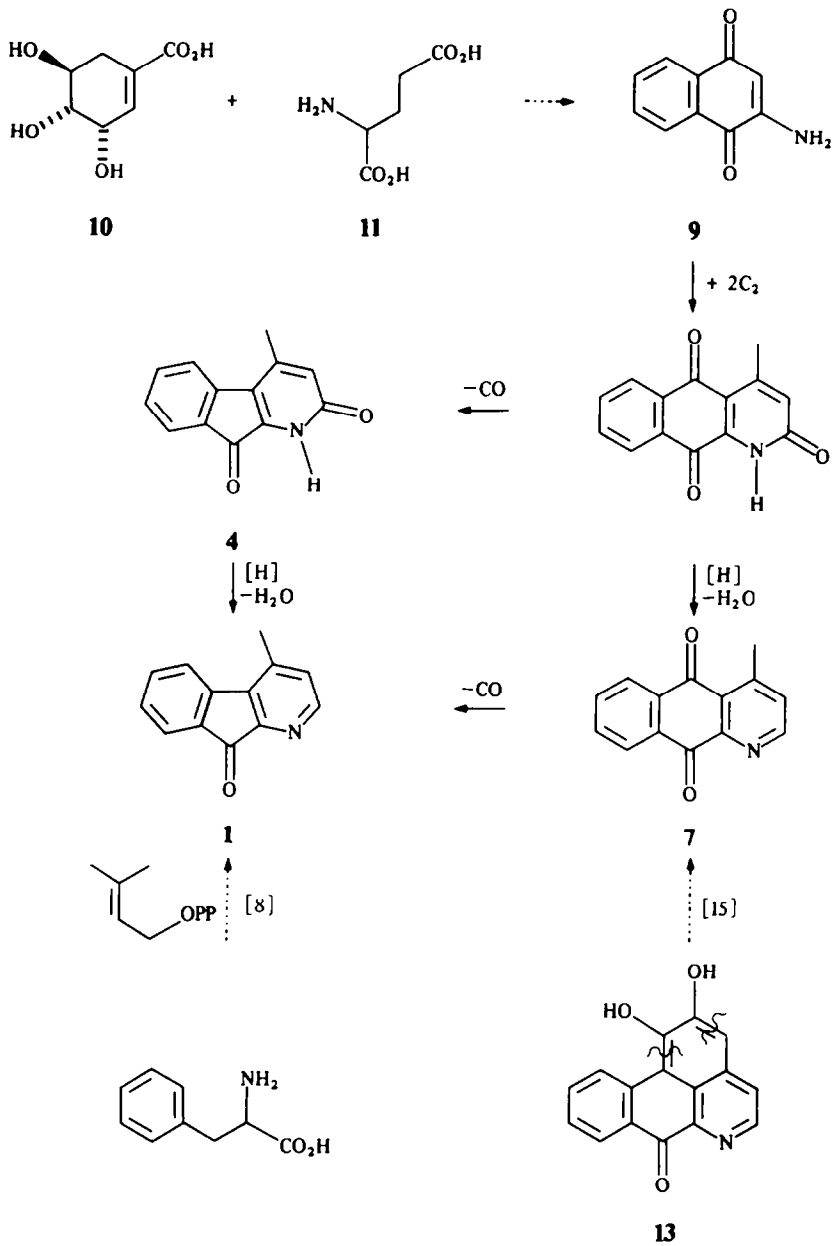
The $CHCl_3$ extract of the HCl insoluble fraction (19.90 g) was chromatographed over silica gel, the column being eluted with solvents of increasing polarity: C_6H_6, $CHCl_3$ and MeOH in different proportions. C_6H_6 eluted seven compounds, in the following order: dielsine (**4**), polycarpol (**3**), dielsiquinone (**6**), *O*-methylmoschatoline, dielsinol (**5**), liriodenine and isomoschatoline. Compound **4** (0.025 g) was isolated by rechromatography over alumina, washing with CCl_4 (to separate the steroid mixture) and recrystallization from Me_2CO. Purification of **3** (0.600 g) involved recrystallization from EtOH. Rechromatography over alumina (C_6H_6–EtOAc, 1:1) and successive washings with Et_2O and EtOAc afforded pure **6** (0.015 g). *O*-Methylmoschatoline (0.015 g), **5** (0.012 g) and liriodenine (0.024 g) were obtained by rechromatography over alumina; the first two compounds were eluted with C_6H_6–EtOAc (9:1) and the third one with C_6H_6–EtOAc (1:1). The last compound, isomoschatoline (0.12 g) was separated by rechromatography over polyamide by elution with C_6H_6–EtOAc (9:1) followed by prep. TLC (C_6H_6–EtOAc–MeOH, 5:4:1) and successive washings with Et_2O.

Onychine (*1-aza-4-methylfluorenone*, **1**). Light yellow needles, mp 125–129°; lit. [8] 133–135°.

6-Methoxyonychine (*1-aza-6-methoxy-4-methylfluorenone*, **2**). Light yellow gum; IR ν_{max}^{film} cm^{-1}: 3420, 2920, 2850, 1700, 1590, 1560, 1465, 1440, 1425, 1375, 1350, 1265, 1255, 1235, 1220, 1210, 1180, 1170, 1140, 1110, 1090, 1050, 1010, 915, 890, 875, 830, 795, 755. UV λ_{max}^{EtOH} nm (log ε): 252 (4.22), 280 (3.79), 292 (3.75), 312 sh (3.43); $\lambda_{max}^{EtOH+HCl}$ nm (log ε): 250 (4.15), 282 sh (3.77), 297 (3.77). EIMS *m/z* (rel. int.): 226 $[M+H]^+$ (10%), 225 $[M]^+$ (100%).

Dielsine (*1-aza-4-methyl-2-oxo-1,2-dihydrofluorenone*, **4**). Yellow needles, mp 254–256° (Me_2CO or HOAc); IR ν_{max} cm^{-1}: 3430, 3190, 1665, 1650, 1595, 1570, 1505, 1475, 1450, 1400, 1375, 1255, 1235, 1205, 1160, 1100, 1080, 1045, 925, 885, 815, 765, 750, 705; UV λ_{max}^{EtOH} nm (log ε): 243 sh (4.15), 256 (4.18), 262 sh (4.17), 272 sh (4.04), 282 (3.56), 341 (3.50); $\lambda_{max}^{EtOH+2.5N\ NaOH}$ nm (log ε): 238 (4.00), 260 sh (4.12), 270 (4.17), 393 (3.87), 489 (3.77). HRMS *m/z* (rel. int.): 212.0661 $[M+H]^+$ (14), 211.0631 $[M]^+$ (100) (calc. for $C_{13}H_9NO_2$: 211.0633), 210.0538 $[M-H]^+$ (64), 183.0648 $[M-CO]^+$ (4), 182.0588 $[M-CHO]^+$ (15), 155.0686 $[M-(2\times CO)]^+$ (11), 154.0648 $[M-H-2\times CO]^+$ (29).

Dielsinol (*1-aza-4-hydroxymethyl-2-oxo-1,2-dihydrofluorenone*, **5**). Yellow greenish crystals, mp 252–254°; IR ν_{max} cm^{-1}: 3400, 3250, 2930, 1655 (bb), 1585, 1505, 1410, 1390, 1300, 1245, 1205, 1160, 1050, 1030, 1020, 930, 715; UV λ_{max}^{EtOH} nm (log ε): 253 (4.26), 269 sh (4.11), 283 (4.03), 333 (3.77); $\lambda_{max}^{EtOH+NaOH}$ nm (log ε): 230 (4.09), 237 sh (4.09),



Scheme 1. Hypothetical biosynthetic pathways to azafluorenones and azaanthraquinones.

260 sh (4.18), 278 (4.24), 380 (3.81). HRMS *m/z* (rel. int.): 227.0579 $[M]^+$ (100) (calc. for $C_{13}H_9NO_3$: 227.0582), 228.0597 $[M+H]^+$ (15), 226.0499 $[M-H]^+$ (25), 225.0424 $[M-2\times H]^+$ (11), 210.0558 $[M-OH]^+$ (12), 209.0483 $[M-H_2O]^+$ (7), 198.0562 $[M-H-CO]^+$ (28), 169.0531 $[M-CH_2O-CO]^+$ (12), 154.0433 $[M-HNCO-CH_2O]^+$ (32).

Dielsiquinone (*1-aza-3-methoxy-4-methyl-2-oxo-1,2-dihydro-9,10-anthracenedione*, **6**). Yellow greenish amorphous powder, mp 250–252° (Me_2CO); IR ν_{max} cm^{-1}: 3280, 2920, 1665, 1655, 1590, 1540, 1480, 1420, 1405, 1320, 1310, 1285, 1270, 1230, 1200, 1120, 1070, 1035, 1015, 970, 800, 780, 725; UV λ_{max}^{EtOH} nm (log ε): 247 sh (4.07), 274 (4.27), 291 (4.25), 322 sh (4.04); $\lambda_{max}^{EtOH+2.5N\,NaOH}$ nm (log ε): 251 (4.23), 270 (4.22), 332 (4.19), 363 sh (4.03); $\lambda_{max}^{EtOH+NaOAc}$ nm (log ε): 250 (4.18), 273 (4.18), 324 (4.17), 361 sh (4.03). EIMS *m/z* (rel. int.): 269 $[M]^+$ (100), 270 $[M+H]^+$ (18), 268 $[M-H]^+$ (30), 254 $[M-Me]^+$ (15), 241 $[M-CO]^+$ (6), 240 $[M-HCO]^+$ (16), 239 $[M-CH_2O]^+$ (8), 105 $[C_7H_5O]^+$ (8).

Polycarpol (*3β,15α-dihydroxy-lanosta-7,9(11),24-triene*, **3**). Colourless amorphous powder, mp 174–177° [EtOH], lit. [9] mp 173–174°. Acetylation with pyridine and Ac_2O afforded in 91% yield colourless needles, mp 123–125°, whose spectral data are consistent with reported data [9]. Oxidation with CrO_3 in Me_2CO [15] afforded lanosta-7,9(11),24-trien-3,15-dione [9] (mp 110–112°), as a colourless amorphous powder.

Isomoschatoline (*2,3-dimethoxy-1-hydroxyaporphine*). Dark red amorphous powder, mp 239–242°. On methylation with CH_2N_2 gave *O*-methylmoschatoline, as yellow needles, mp 178–180° (Et_2O), lit. [10] mp 177° (Et_2O).

Acknowledgements—The authors are grateful to Prof. A. Cavé, Faculté de Pharmacie, Chatenay-Malabry (France) for a gener-

ous gift of an authentic sample of polycarpol. ^{1}H NMR spectra at 250 and 400 MHz were obtained in France, by C. Mérienne (Université de Paris-Sud, Orsay) by courtesy of Dr. G. Lukacs (ICSN-CNRS, Gif-sur-Yvette) to whom we are grateful. We also wish to thank Dr. P. G. Waterman (University of Strathclyde, Glasgow) for supplying us with part of ref. [14] before its publication. Fellowships by CAPES (Goulart, M. O. F. and Sant'Ana, A. E. G.) and CNPq (Oliveira, A. B., Oliveira, G. G. de and Maia, J. G. S.) are gratefully acknowledged. This work was supported by CNPq and FINEP.

REFERENCES

1. Walker, J. M. (1971) *Contributions Gray Herbarium* 202.
2. Thorne, R. F. (1974) *Aliso* **8**, 147.
3. Hegnauer, R. (1964) *Chemotoxonomie der Pflanzen*, Vol. 3, p. 116. Birkhauser, Basel.
4. Leboeuf, M., Cavé, A., Bhaumik, P. K., Mukherjee B. and Mukherjee, R. (1982) *Phytochemistry* **21**, 2783.
5. Shamma, M. and Guinaudeau, H. (1984) *Nat. Prod. Rep.* **1**, 201.
6. Waterman, P. G. (1984) *Rev. Latinoam. Quim.* **15**, 90.
7. Waterman, P. G. and Muhammad, I. (1985) *Phytochemistry* **24**, 523.
8. De Almeida, M. E. L., Braz Fo, R., von Bulow, M. V., Gottlieb, O. R. and Maia, J. G. S. (1976) *Phytochemistry* **15**, 1186.
9. Hamonnière, M., Fournet, A., Leboeuf, M., Bouquet, A. and Cavé, A. (1976) *C. R. Acad. Sci. Paris Ser. C* **282**, 1045.
10. Dwuma-Badu, M., Aym, J. S. K., Tachie, A. N., Knapp, J. E., Slatkin, D. J. and Schiff, P. L., Jr. (1975) *Phytochemistry* **14**, 2524.
11. Atti, S. A., Ammar, H. A., Phoebe, C. H., Jr., Schiff, P. L., Jr. and Slatkin, D. J. (1982) *J. Nat. Prod.* **45**, 476.
12. Thomson, R. H. (1971) *Naturally Occurring Quinones*. Academic Press, London.
13. Nadzan, A. M. and Reinehart, R. L. (1976) *J. Am. Chem. Soc.* **98**, 5012.
14. Cavé, A., Leboeuf, M. and Waterman, P. G. (1986) in *Alkaloids: Chemical and Biological Perspectives* (Pelletier, S. W., ed.) Vol. 5. John Wiley, New York.
15. Bowers, A., Halsall, T., Jones, E. and Lemin, A. (1953) *J. Chem. Soc.* 2548.