*Phytochemistry*, Vol. 28, No. 1, pp. 231-234, 1989.
Printed in Great Britain.

0031-9422/89 $3.00+0.00


# PACHYPOPHYLLIN AND PACHYPOSTAUDINS A AND B: THREE *BISNOR*LIGNANS FROM *PACHYPODANTHIUM STAUDTII**

BONAVENTURE T. NGADJUI, DAVID LONTSI, JOHNSON FOYERE AYAFOR* and B. LUCAS SONDENGAM

Department of Organic Chemistry, Faculty of Sciences, The University of Yaoundé, Box 812, Yaoundé, Cameroon

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

**Key Word Index**—*Pachypodanthium staudtii*; Annonaceae; *bisnor*lignans; pachypophyllin; pachypostaudin-A; pachypostaudin-B.

**Abstract**—Three new *bisnor*lignans, pachypophyllin, pachypostaudin-A and pachypostaudin-B have been isolated from the bark of *Pachypodanthium staudtii* and their structures have been determined by $^1$H and $^{13}$C NMR spectroscopy and chemical correlations. 2,4,5-Trimethoxystyrene, probably a precursor of the *bisnor*lignans, was also isolated from the extracts. Furthermore, the potent antiviral flavonol, pachypodol, was obtained in significant amount.

## INTRODUCTION

*Pachypodanthium staudtii* Engl. & Diels is a small tree which grows in the tropical forest zone of West and Central Africa. Various parts of the plant are a popular remedy for chest pain, bronchitis, gastro-intestinal troubles, oedemas and cancer [1, 2]. *Pachypodanthium staudtii* is known for elaborating a number of aporphine alkaloids [3] as well as isoquinolines [3], protoberberines [3], flavonoids [3] and varied benzene derivatives [4]. Our interest in the systematic study of the chemical constituents of Cameroonian medicinal plants has led us to the investigation of the stem bark of *Pachypodanthium staudtii*. We now report on the contents of the hexane extracts of this plant. The potent antiviral agent, 3,7,3′-trimethylquercetin (pachypodol) [5] was obtained along with three novel unusual aryltetralin norlignans, pachypostaudin-A (**2**), pachypostaudin-B (3), and pachypophyllin (4). The benzene derivative, 2,4,5-trimethoxystyrene (**1**) [4], a probable congener of the norlignans was qlso isolated. Whereas **1** occurs in great quantities in the families Piperaceae and Annonaceae [3], 3,7,3′-trimethylquercetin is rare and has been previously reported only in trace amounts from *Euphorbia grantii* [5] and *Pachypodanthium confine* [3].

## RESULTS AND DISCUSSION

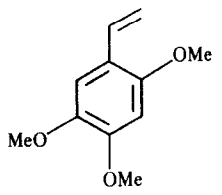
The finely powdered stem bark of *Pachypodanthium staudtii* was extracted successively with *n*-hexane, chloroform and methanol. Fractionation of the concentrated *n*-hexane extract on silica gel (see Experimental) yielded several fractions which on further preparative chromatographic purification afforded 2,4,5-trimethoxystyrene (**1**), pachypostaudin-A (**2**), pachypostaudin-B (**3**), and pachypophyllin (**4**).

Compound **1** (mp 60°, $M_r$ 194) was identified as the known 2,4,5-trimethoxystyrene by direct comparison of its physical and spectral data (mp, IR, MS, $^1$H NMR) with an authentic specimen and published data [6].

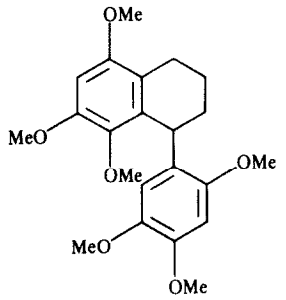
Compound **2**, (mp, 130°) provisionally named pachypostaudin-A, was unknown in the literature. Its molecular composition was found to be $C_{22}H_{28}O_6$ by high resolution mass spectroscopy. The UV spectrum of **2** showed maxima at $\lambda_{max}$ 225 and 285 nm characteristic of a benzene chomophore substituted by methoxyl groups [7]. The IR spectrum showed strong vibrations at $\nu_{max}$ 2800, 1300, 1225 and 1070 cm$^{-1}$ indicative of the presence of methoxyl groups and 1600, 1580 and 1500 cm$^{-1}$ confirming the existence of benzene rings. The $^1$H NMR spectrum contained signals for three methylene groups ($\delta$ 1.5–2.8), six methoxyl groups [$\delta$ 3.32 (3H, *s*), 3.62 (3H, *s*) and 3.90 (12H, *s*)], one benzylic methine proton at $\delta$4.78 and three aromatic hydrogens ($\delta$6.25, 6.48 and 6.60). The EI mass spectrum showed the molecular ion $M^+$ and *m/z* 388 which was also the base peak and significant fragmentation peaks at *m/z* 373 $[M-Me]^+$, 358 $[M-2\times Me]^+$, 357 $[M-OMe]^+$, 326 $[M-2\times OMe]^+$, 220, 194 and 167. The peak at *m/z* 194, which is the most important after the base peak, confirmed the presence of a 2,4,5-trimethoxystyrenyl moiety that might be a congener of **2** (*vide infra*). The above data, coupled with the proper spin decoupling experiments in the $^1$H NMR spectrum, suggested structure **2** for pachypostaudin-A. This structure was further confirmed by the $^{13}$C NMR data which showed six aromatic C–O singlets ($\delta$ 153.3, 151.0, 150.6, 147.7, 142.3 and 140.8), six aromatic C–C, three as doublets ($\delta$115.5, 98.3, and 95.7) and three as singlets ($\delta$134.3, 128.1 and 119.6). The six methoxyl groups resonated at $\delta$60.0, 57.2, 56.9, 56.6, 56.3 and 55.6, respectively, while the methine carbon appeared as a doublet at $\delta$31.9. Finally, the chemical shifts of the methylene carbons were observed as triplets at $\delta$29.1, 22.9 and 17.5, respectively.

The third compound pachypostaudin-B (**3**) had two hydrogen atoms less in the molecular formula,

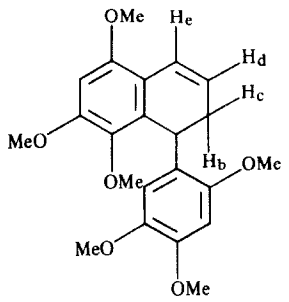
*A part of this work was presented at the International Foundation for Science, Seminar on Pharmacological Screening of Medicinal Plants, Harare, December 1986.



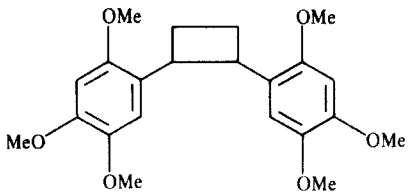

1



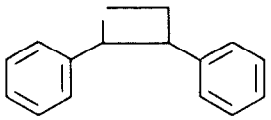

2



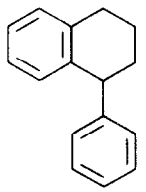

3



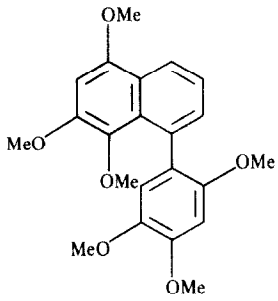

4



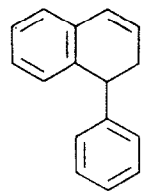
6



7




5



8

$C_{22}H_{26}O_6$, and crystallized as needles from hexane–ethyl acetate, mp 120°. Its structure could be deduced from the $^1H$ and $^{13}C$ NMR spectra (Experimental) which were in part similar to those of pachypostaudin-A (**2**). In particular, the proton resonances for the six methoxyl groups and the three aromatic protons in **3** were almost superimposable on the corresponding signals in the spectrum of **2**. On the other hand, the most significant differences in the $^1H$ and $^{13}C$ NMR spectra of **2** and **3** were found in the aliphatic region where the three methylene proton signals in **2** and $\delta$ 1.5–2.0 (*m*, 4H), 2.5–2.5 (*br m*, 2H), 29.1 (*t*), 22.9 (*t*) and 17.5 (*t*) were replaced by resonances for a disubstituted double bond [$\delta$6.8 (1H, *dd*, $J = 10$; 2Hz, $H_e$), 5.7 (1H, *ddd*, $J = 5$, 10, 2 Hz, $H_d$), 123.5 (*d*) and 121.2 (*d*)] and a single methylene group [$\delta$2.55 (2H, *m*, $H_b$ and $H_c$); 29.8 (*t*)]. Catalytic hydrogenation of **3** yielded a product which was identical in all respects to **2** confirming the fact that pachyposdautin-A (**2**) was a dihydroderivative of pachypostaudin-B (**3**).

Therefore, there remained only the position of the double bond to be determined. Spin decoupling experiments in the $^1H$ NMR spectrum of **3** were useful in this respect. Thus, irradiation of the two proton multiplet ($H_2$-2) at $\delta$2.55 reduced the doublet of doublets at $\delta$6.8 (H-4) and the double doublets at 5.7 (H-3) to an AB quartet ($J = 10$ Hz) and also collapsed the doublet of doublets at 4.9 (H-1). The above data, coupled with chemical shifts, located the double bond unambiguously between C-3 and C-4. The position of the double bond was further confirmed by the UV spectrum of **3** which showed a bathochromic shift of $\Delta$ 10 nm compared to that of **2** indicating the extension of the benzene chromophore. Thus, structure **3** was assigned to pachypostaudin-B.

A few reactions were undertaken on pachypostaudin-A (**2**) and B (**3**) to further confirm their structures. As already mentioned above, catalytic hydrogenation of pachypostaudin-B (**3**) over Pd/C gave pachypostaudin-A (**2**) while

dehydrogenation of both **2** and **3** with DDQ in refluxing benzene afforded the naphthalene derivative **5** whose structure followed from its spectroscopic data. The naphthalene, mp 125–127°, $C_{22}H_{24}O_6$ from high resolution EI mass spectrometry, showed characteristic UV absorptions at $\lambda_{max}$ 246 (log $\varepsilon$ 4.40), 310 (4.05) and 340 (3.18) nm. The EI mass spectrum of **5** showed $M^+$ at *m/z* 384 which was also the base peak, and other major fragments at *m/z* 338, 323 and 192. The $^1$H NMR spectrum was well-resolved and revealed resonances for six methoxyl groups [$\delta$3.17 (3H, *s*), 3.62 (3H, *s*), 3.93 (6H, *s*) and 3.99 (3H, *s*)]. The strongly shielded methoxyl ($\delta$3.17) was assigned to C-8. Its relatively higher shielding compared to that in pachypostaudin-A (**2**) ($\delta$3.32) and in pachypostaudin-B (**3**) ($\delta$3.40) is probably due to the relative orientation of the 2,4,5-trimethoxyphenyl moiety at C-1 of the naphthalene which is presumably perpendicular to the mean plane of the naphthalene. Because of this orientation, the methoxyl group at C-8 should be fully shielded by the anisotropic effect of the benzene ring at C-1. Six aromatic protons were also identifiable in the $^1$H NMR spectrum, three as one proton singlets and the other three as a three-proton multiplet.

Pachypophyllin (**4**), $C_{22}H_{28}O_6$, crystallized from ether–petrol as prisms, mp 158°. The EI mass spectrum of **4** gave a small parent peak $M^+$ at *m/z* 388 and major fragments at *m/z* 194, 179, 151, 136 and 91. The mass of the base peak (194 amu) is exactly half of pachypophyllin and corresponds to 2,4,5-trimethoxystyrene, the major constituent of *P. staudtii*. Therefore **4** could be another dimer of 2,4,5-trimethoxystyrene.

The $^{13}$C NMR spectrum showed only eleven resonances (see Experimental). It was apparent therefore that pachypophyllin was a symmetrical dimer of 2,4,5-trimethoxystyrene. The presence of a cyclobutane ring follows from the fact that the non-aromatic protons (apart from the methoxyl groups) are a methine and a methylene, respectively. Furthermore, the complexity of the methylene proton signals suggests a 1,2-rather than a 1,3-substitution pattern for the cyclobutane ring.

The 200 MHz $^1$H NMR spectrum of **4** is also consistent with the proposed structure. Of particular interest in this spectrum are the two upfield complex multiplets of the protons of the two methylene groups at $\delta$1.95 (2H) and 3.31 (2H). The complexity of these signals even after irradiating at the frequency of the hidden methine resonances at $\delta$ *ca* 3.85) lends further support for the 1,2-substitution pattern of the cyclobutane ring. Pachypophyllin was thus proposed to have structure **4**. The relative stereochemistry however remains to be determined.

It is interesting to note that pachypostaudin-A (**2**), pachypostaudin-B (**3**) and pachypophyllin (**4**) are racemic. The formation of **2** and **3** can be rationalized in terms of non-enzymatic controlled Diels–Alder dimerization of 2,4,5-trimethoxystyrene (**1**) followed by aromatization and, in the case of **3**, by dehydrogenation. The major constituents (**1**–**4**) of the hexane extracts of the stem bark of *P. staudtii* are easily revealed on a TLC plate (silica gel) by spraying with Dragendorff reagent and the fact that all the four spots are conspicuous even in the cold hexane extracts of *P. staudtii* is clear evidence that **2**–**4** are not artefacts formed during the isolation procedure.

Pachypostaudin-A (**2**), pachypostaudin-B (**3**) and pachypophyllin (**4**) can therefore be considered to belong to a new class of naturally occurring *bisnor*lignans. It is however worthwhile mentioning that **2**–**4** are closely related to **6**, **7** and **8** obtained by Mayo [7] along with higher polymers during the thermal polymerization of styrene.

Finally, attempts to synthesize the *bisnor*lignans by thermolysis [8] (see Experimental) on the one hand and by irradiation on the other hand of 2,4,5-trimethoxystyrene (**1**) were unsuccessful.

Our sample of 3,7,3′-tri-*O*-methylquercetin showed antiviral activity against the polio virus at a concentration of 1–50 $\mu$g/ml but was inactive against *Herpes simplex* type 1 virus. These preliminary results are similar to those previously published by Vanden Berghe and co-workers who demonstrated that 3-methoxyflavones in general act as potent inhibitors of the viral induced block of cell synthesis [5].

## EXPERIMENTAL

*General.* Mps: uncorr. UV spectra were obtained in MeOH and IR spectra in KBr pellets unless otherwise stated. $^1$H NMR spectra were recorded at 80 and 200 MHz in $CHCl_3$ with TMS as int. standard; chemical shifts are reported in $\delta$ units. High resolution EIMS and low-resolution EIMS: 70 eV, direct probe insert between 120 and 150°. The *bisnor*lignans were visualized by spraying with Dragendorff reagent.

*Plant material*, the stem bark of *P. staudtii* used in this study was collected at the Edea forest Reserve during June–July 1984. The identification was confirmed by Mr Benoît Mpom of the Cameroon National Herbarium, Yaounde, where a voucher specimen is kept.

*Extraction and isolation of the* bisnor*lignans.* The powdered trunk bark (5 kg) was successively extracted with hexane, $CHCl_3$ and MeOH. The $CHCl_3$ and MeOH extracts constitute a separate study. Concentration of the hexane extract under red. pres. (50°) afforded a brownish semi-solid (152 g). Part of this material (50 g) was fractionated over silica gel (800 g) packed in hexane and eluted with hexane–EtOAc mixture, 250 ml fractions were collected.

2,4,5-*Trimethoxystyrene* (**1**). Eluted by hexane–EtOAc (9:1) and crystallized from hexane–EtOAc as cream-coloured prisms, (33 g), mp 64° (lit. [6] 60°), identical by direct comparison (UV, IR, $^1$H NMR, MS) with an authentic sample and lit. data [6].

*Pachypostaudin-A* (**2**). Eluted with hexane–EtOAc (7:3); colourless granules from the same solvent mixture (120 mg), mp 130° $[\alpha]_D^{24}$ 0 ($CHCl_3$; *c* 0.8); IR $\nu_{max}$ cm$^{-1}$: 2900, 1600, 1580 and 1500 (aromatic system); UV $\lambda_{max}^{MeOH}$ nm: 225 and 285; $^1$H NMR ($CDCl_3$, 80 MHz); $\delta$1.50–2.00 (4H, *br m*), 2.5–2.8 (2H, several multiplets), 3.32 (3H, *s*, OMe), 3.62 (3H, *s*, OMe), 3.90 (12H, *s*, 3 × OMe), 4.78 (1H, *m*, H-1). 6.25 (1H, *s*, Ar-H), 6.48 (1H, *s*, Ar-H), and 6.60 (1H, *s*, Ar-H); $^{13}$C NMR (partial assignments only): $\delta$ 153.3 *s*, 154.0 (*s*), 150.6 (*s*), 147 (*s*), 142.3 (*s*) and 140.8 (*s*) (six methoxylated carbons), 134.3 (*s*), 128.1 (*s*), 119.6 (*s*) (three aromatic carbons), 115.5 (*d*), 98.5 (*d*) and 95.7 (*d*) (aromatic methines), 60.0 *q*, 57.2 (*q*), 56.9 (*q*), 56.6 (*q*), 56.3 (*q*) and 55.6 (*q*) (six methoxyl carbons), and 31.9 (*d*, C-1), 29.1 (*t*), 22.9 (*t*) and 17.5 *t* (aliphatic carbons). Found: $[M]^+$ at *m/z* 388.1884; $C_{22}H_{28}O_6$ requires 388.1886. HREIMS *m/z* (rel. int.): 220.1094 $[C_{13}H_{16}O_3]^+$ (20), 194.0948 $(C_{11}H_{14}O_3]^+$ (43), 167.0713 $[C_9H_{11}O_3]^+$ (4), and 151.0764 $[C_9H_{11}O_2]^+$ (7).

*Pachypostaudin-B* (**3**). Colourless needles from hexane–EtOAc (95 mg) mp 120°, $[\alpha]_D^{24}$ 0 ($CHCl_3$; *c* 0.1). UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 235 (4.13) and 295 (4.02): $^1$H NMR ($CDCl_3$, 80 MHz): $\delta$ 2.55 (2H, *m*, 2H-2), 3.40 (3H, *s*, OMe-8), 3.60 (3H, *s*, OMe), 3.90 (12H, *s*, 4 × OMe), 4.90 (1H, *dd*, *J* = 2, 8 Hz, H-1), 5.70 (1H, *ddd*, *J* = 2, 5, 10 Hz, H-3), 6.30 (1H, *s*, Ar-H), 6.50 (1H, *s*, Ar-H), 6.60

(1H, s, Ar-H) and 6.80 (1H, dd, $J=2$, 10 Hz, H-4); $^{13}$C NMR (25.2 MHz, $CHCl_3$): 152.5 (s), 151.6 (s), 150.7 (s), 148.0 (s), 142.5 (s), 123.5 (d, C-4), 121.2 (d, C-3), 114.5 (d), 98.1 (d), 96.1 (d) (three aromatic methines), 60.4 (q), 56.9 (q), 56.7 (q), 56.3 (1), 56.1 (q), 55.9 (q) (6 × OMe), 29.8 (t, C-2) and 28.4 (d, C-1). Found $[M]^+$ at $m/z$ 386.1740. $C_{22}H_{26}O_6$ requires 386.1741. EIMS $m/z$ (rel. int.): 355 $[C_{21}H_{23}O_5]^+$ (4), 218 $[C_{13}H_{14}O_3]^+$ (4), 194 $[C_{11}H_{14}O_3]^+$ (100), 151 $[C_9H_{11}O_2]^+$ (6) and 136 $[C_8H_8O_2]^+$ (2).

*Pachypophyllin* (**4**). Colourless prisms from hexane–EtOAc, (800 mg) mp 158°; $[\alpha]_D^{24}$ 0 (*c*. 0.8; $CHCl_3$) UV $\lambda_{max}^{MeOH}$ nm (log ε): 225 (4.10) and 285 (3.85); $^1$H NMR ($CHCl_3$, 300 MHz): δ 1.95 (2H, *m*), 2.31 (2H, *m*), 3.74 (6H, *s*, 2 × OMe), 3.83 (6H, *s*, 2 × OMe), 3.85 (6H, *s*, 2 × OMe), *ca* 3.85 (obscured 2H, *m*), 6.47 (2H, *s*, 2 × Ar-MH) and 6.97 (2H, *s*, 2 × Ar-H); $^{13}$C NMR: only 11 out of the 22 carbon atoms observed at δ (151.3 (*s*), 147.8 (*s*), 143.3 (*s*) (six methoxylated carbons), 124.8 (*s*), 112.1 (*d*), 98.0 (*d*), 56.7 (*q*), 56.8 (*q*), 56.3 (*q*), 40.6 (*d*) and 27.1 (*t*, 2 × $CH_2$). Found 388.1886; $C_{22}H_{28}O_6$ requires 388.1884; EIMS $m/z$ (rel. int.): 388 $[M]^+$ (0.6), 194 (100), 179 $[C_{10}H_{11}O_3]^+$ (8), 151 $[C_9H_{11}O_2]^+$ (4), 136 $[C_8H_8O_2]^+$ (2) and 91 $[C_7H_7]^+$ (2).

*Interconversion of pachypostaudin-A and pachypostaudin-B.* Pachypostaudin-B (20 mg) in EtOAc (5 ml) was shaken with 5% Pd/C in a hydrogen atmosphere for 30 min. Filtration and evapn of the solvent afforded pachypostaudin-A (18 mg) which was crystallized from hexane–EtOAc and mp UV, IR and $^1$H NMR identical with that of the natural sample.

*Dehydrogenation of pachypostaudin-A.* Pachypostaudin-A (10 mg) and DDQ (10 mg) were dissolved in benzene (5 ml) and heated under reflux for 3 hr. Removal of solvent followed by prep. TLC afforded the naphthalene derivative **5** (3 mg); mp 125–127°; (from $CHCl_3$–hexane); UV $\lambda_{max}^{MeOH}$ nm (log ε): 246 (4.40), 310 (3.85) and 340 nm (sh); IR $\nu_{max}^{CCl_4}$ $cm^{-1}$: 2990, 2930, 2810, 2830, 1610, 1505, 1450, 1440, 1390, 1360, 1330, 1255, 1205, 1175, 1160, 1150, 1130, 1070, 1090, 980, 960, 915, 860, 830, 795 and 730; $^1$H NMR ($CDCl_3$, 80 MHz): δ 3.17 (3H, *s*, OMe), 3.82 (3H, *s*, OMe), 3.60 (3H, *s*, OMe), 3.93 (6H, *s*, 2 × OMe), 3.99 (3H, *s*, OMe), and 7.29 (3H, *m*, Ar-H); HREIMS $m/z$ (rel. int.): 384.1585 $[M]^+$ ($C_{22}H_{24}O_6$) (100), 385 $[M+1]^+$ (79), 339.1178 $[C_{11}H_{12}O_3]^+$ (12), 169.0579 (20).

*Attempted dimerization of* 2,4,5-*trimethoxystyrene* (**1**). 2,4,5-Trimethoxystyrene (1 g) was dissolved in bromobenzene (7 ml) with iodine (5 mg) as catalyst. This was refluxed in an atmosphere of nitrogen for 24 hr at approximately 150°. The soln was then distilled in a Kugelrohr and heated to 150° at 1 mm Hg when it immediately solidified due to polymerization. TLC analysis of the reaction mixture showed no trace of the *bisnor*ligans **2**, **3** or **4**.

*Irradiation of* 2,4,5-*trimethoxystyrene* (**1**). 2,4,5-Trimethoxystyrene (0.5 g) was dissolved in EtOAc (200 ml) with $Me_2CO$ (2 ml). The soln was then irradiated in a quartz apparatus using a medium pressure Itanovia lamp (125 w) over a 24 hr period and the reaction monitored by TLC. After this period, the reaction mixture was evaporated to leave a brown oil which was shown by TLC to be the starting material.

3,7,3′-*Trimethylquercetin.* Eluted by hexane-$CHCl_3$ (2:3). (1.80 g) yellow needles from hexane-EtOAc, mp 171–172° (lit. [5] 169°). IR, UV, EIMS and $^1$H NMR spectra were identical to those reported for pachypodol [9].

*Acknowledgements*—The authors are grateful to Dr J. D. Connolly (University of Glasgow) for the MS and the $^1$H and $^{13}$C NMR spectral data and for very fruitful discussions. Financial support for this work by the International Foundation for Science (Stockholm) through IFS grant No. F/933-1 to B.T.N. is also gratefully acknowledged.

## REFERENCES

1. Burkill, H. M. (1985) *The Useful Plants of West Tropical Africa*, Vol. 1. 2nd Edn. Royal Botanic Garden, Kew.
2. Bouquet, A. (1969) *Féticheurs et Médécines du Congo (Brazzaville)* ORSTOM, Paris.
3. Leboeuf, M. Cavé, A., Bhaumik, P. K., Mukherjee, B. and Mukherjee, R. (1982) *Phytochemistry* **21**, 2783.
4. Bevalot, F. Leboeuf, M. and Cavé, A. (1978) *C.R. Acad. Sci. Paris Ser. C.* **286**, 405.
5. Van Hoof, A., Vanden Berghe, D. A. Hartfield, G. M. and Vlientinck, A. (1984) *Planta Med.* **50**, 513.
6. Waterman, P. G. (1976) *Phytochemistry* **15**, 347.
7. Mayo, F. R. (1968) *J. Am. Chem. Soc.* **90**, 1289.
8. Dodson, R. M. and Zielske, A. G. (1967) *J. Org. Chem.* **32**, 28.
9. Cavé, A. Bouquet, A. and Paris, R. R. (1973) *C.R. Acad. Sci. Paris, Ser. D.* **276**, 1899.