Pergamon

0031-9422(94)00598-2

Phytochemistry, Vol. 38, No. 2, pp. 477–480, 1995
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0031-9422/95 $9.50 + 0.00

BIOACTIVE ANTHRAQUINONE GLYCOSIDES FROM *PICRAMNIA ANTIDESMA* SSP. *FESSONIA*

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(*Received* 15 *June* 1994)

Key Word Index—*Picramnia antidesma* ssp. *fessonia*; Simaroubaceae; anthraquinones; novel glycosides; KB cell; brine shrimp; picramniosides A, B, C.

Abstract—A bioactivity guided fractionation, using KB cells and brine shrimp assays, of the methanolic extract from the leaves of *Picramnia antidesma* yielded two known anthraquinones, aloe-emodin and aloe-emodin anthrone, and three new aloe-emodin *C*-glycosides, named picramnioside A, picramnioside B and picramnioside C. Structures were established by spectroscopic methods (UV, IR, mass spectrometry, ^{1}H and ^{13}C and 2D NMR including COSY 45, HMQC, HMBC and ROESY). CD was used to establish the absolute configuration of the picramniosides.

INTRODUCTION

Picramnia antidesma ssp. *fessonia* (DC.) W. Thomas is the most common and variable taxon of *Picramnia* in Mexico and Central America [1]. It is a small tree member of the Simaroubaceae, a family known to contain quassinoids with antiplasmodial [2] and cytotoxic activities [3]. We decided to investigate this plant because of our interest in antiprotozoal natural products and the possibility of obtaining different quassinoids.

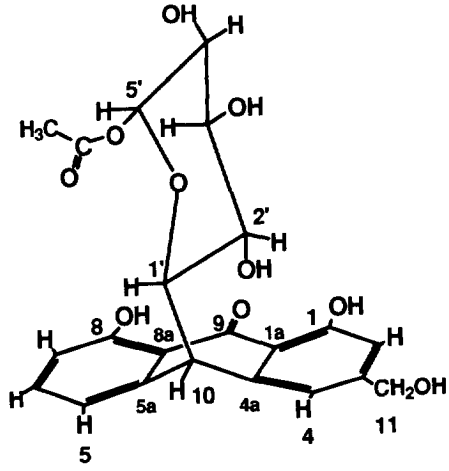
This species has been used as a vermicide in Jamaica [4] and in Mexico it is said to be poisonous [5]. A chloroformic extract has been shown to be active against *Plasmodium gallinaceum, in vivo* [6] and sitosterol is the only compound previously reported from this plant [5]. There have been several previous reports of quinoids [7–9] and triterpenoids [10] in the genus *Picramnia*.

RESULTS AND DISCUSSION

A bioactivity guided fractionation of the methanolic extract from *P. antidesma* yielded two known anthraquinones, aloe-emodin and aloe-emodin anthrone, and three new aloe-emodin *C*-glycosides, named picramnioside A (**1**), picramnioside B (**2**) and picramnioside C (**3**). This plant was selected because of its activity against *P. gallinaceum* [6] and from the chemotaxonomic standpoint the presence of quassinoids was predictable. Only the butanolic fraction (see Experimental) proved to be active against KB cell (LC_{50} 8.7 $\mu g\,ml^{-1}$); however, this fraction showed no significant activity against brine shrimp (LC_{50}485 $\mu g\,ml^{-1}$).

The UV spectrum of **1** showed four bands (221, 273, 302 and 382 nm) characteristic of a highly conjugated system, such as an anthraquinone and the IR spectrum showed a band at 1725 cm^{-1} for a ketone group. The FDMS showed a strong peak at *m/z* 531 (100) $[M + Na]^+$ and *m/z* 508 (12) for $[M]^+$, and FAB (glycerol/thioglycerol/TFA matrix) showed a weak $[M + 1]^+$ peak at *m/z* 509 (17). The peaks at *m/z* 387 (54) indicated the loss of a benzoate moiety $[M - 122]^+$ and at *m/z* 256 indicated the aglycone. HRMS (FAB) showed a $[M - benzoate]^+$ *m/z* 387.0716 corresponding to the molecular formula $C_{20}H_{19}O_8$; the peak at *m/z* 509 was too small to be measured using this technique.

^{1}H NMR of **1** showed two singlets at δ11.96 and 11.83 assigned to the OH groups on C-1 and C-8; there were signals for 10 aromatic protons from δ6.63 to 7.72, and the COSY 45 spectrum showed three separated aromatic rings; a singlet at δ5.62 was assigned to the proton on C-5′; four D_2O exchangeable protons (δ5.41, 5.20, 5.07 and 4.91) were assigned to the hydroxyl groups in the sugar moiety and to the hydroxyl on C-11 in the aglycone; a double doublet at δ4.65 was assigned to the proton on C-1′; the doublet intergrating for two protons at δ4.02 was assigned to the protons on C-11; a doublet of doublets at δ3.85 accounted for the proton on C-1′ of the sugar, showing a $J_{1'-10} = 2.1$ and $J_{1'-2'} = 9.9$, suggesting a β configuration for the carbon of the sugar bound to the aglycone, close to those reported for aloins [11]; the two proton singlet-shaped multiplet at δ2.90 was assigned to the protons on C-3′ and C-4′ and a multiplet at δ3.53–3.44 accounted for the proton on C-2′. The ^{13}C NMR assignment is presented in Table 1. The spectrum showed 25 signals, including two CH signals at δ129.2 and 133.7 showing a double intensity characteristic of a monosubstituted benzene ring. An interesting



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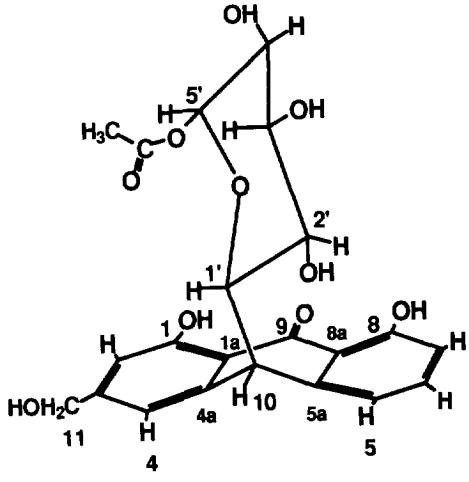

3

Table 1. ^{13}C NMR spectral data* of the compounds isolated from *P. antidesma* ssp. *fessonia*

C	1	2	3
1	161.4 (C)†	161.2 (C)†	161.2 (C)†
1a	115.6 (C)	115.7 (C)	115.4 (C)
2	112.2 (CH)	113.0 (CH)	112.1 (CH)
3	152.6 (C)	151.8 (C)	152.3 (C)
4	115.7 (CH)	118.4 (CH)	115.5 (CH)
4a	141.3 (C)	141.2 (C)	141.1 (C)
5	120.7 (CH)	118.2 (CH)	120.4 (CH)
5a	146.0 (C)	146.1 (C)	145.8 (C)
6	135.8 (CH)	136.3 (CH)	135.4 (CH)
7	116.1 (CH)	115.7 (CH)	115.8 (CH)
8	161.5 (C)	161.6 (C)	161.2 (C)
8a	117.2 (C)	117.2 (C)	116.9 (C)
9	193.5 (C)	193.5 (C)	193.2 (C)
10	42.4 (CH)	42.7 (CH)	42.3 (CH)
11	62.1 (CH_2)	62.6 (CH_2)	62.3 (CH_2)
1′	81.6 (CH)	80.9 (CH)	80.7 (CH)
2′	66.8 (CH)	66.7 (CH)	66.5 (CH)
3′	71.9 (CH)	71.7 (CH)	71.4 (CH)
4′	69.4 (CH)	69.3 (CH)	69.0 (CH)
5′	94.7 (CH)	93.7 (CH)	93.4 (CH)
5′ (C = O)	163.4 (C)	168.0 (C)	167.7 (C)
5′ (CH_3)	—	20.5 (Me)	20.1
1″	129.2 (C)	—	—
2″/6″	129.2 (CH)‡	—	—
3″/5″	128.9 (C)‡	—	—
4″	133.7 (CH)	—	—

*Chemical shifts are in δ-values in ppm, measured in DMSO-d_6.

†Multiplicity from Dept ^{13}C NMR experiment.

‡The intensity for this signal was twice that of the other CH signals.

feature of this *C*-glycoside in ^{13}C NMR is the low field position of the signal for C-5′ (δ94.7), which may indicate the presence of an *O*-glycoside. In fact, this sugar residue has the same configuration of xylose indicated by ROESY NMR experiment, but, it has the aloe-emodinanthrone moiety attached to the C-1 and the benzoate is attached to C-5 of the xylose moiety.

2D NMR experiments such as COSY 45, HMQC, HMBC and ROESY were used to confirm these assignments. The HMBC NMR experiment showed that the singlet at δ 5.62 corresponding to the proton on C-5′ and the double doublet at δ7.73–7.65 assigned to the protons on C-2″ and C-6″, were correlating to the carbon at δ163.4 assigned to the carbonyl of the ester, thus establishing the position of the benzoate on C-5′. Also, the signal at δ4.65 for the proton on C-10 showed a correlation to the carbon at δ81.6 for the C-1′, corroborating the position of attachment between the aglycone and the sugar moiety. In addition, the ROESY NMR experiment showed a correlation between the signal at δ5.62 for the proton on C-5′ and the signals at δ3.85, 3.67 and 5.01 for the protons on C-1′, C-3′ and the OH on C-4′, respectively; indicating that all the substituents on the sugar moiety are in the same configuration. Moreover, the proton on C-1′ (δ3.85) and the signal for the proton on C-4 (δ6.83) showed correlation, as did the multiplet at δ3.45–3.53 (H-2′) and the doublet at δ7.15 (H-5). Therefore the configuration of the C-10 is *R*, in agreement of the data previously reported for aloins [11] and cascarosides [12]. The ROESY effects displayed for **1** and its absolute configuration are shown in Figure 1. Also, the circular dichroism agreed with that previously reported for (10*R*) aloin, showing a negative Cotton effect at 295.0 nm [11].

The UV spectra of **2** and **3** were similar and the IR spectra showed few differences; the carbonyl band which appeared at 1725 cm^{-1} in the spectrum of **2** was at 1760 cm^{-1} in **3**. Oxidative hydrolysis [13] of both compounds yielded aloe-emodin, which was identified by comparison of TLC and ^{1}H NMR with a reference sample. FAB (glycerol/thyoglycerol/TFA matrix) showed, for both **2** and **3**, a $[M + 1]^+$ peak at *m/z* 447, a peak at *m/z* 387 indicating the loss of the acetate of the sugar

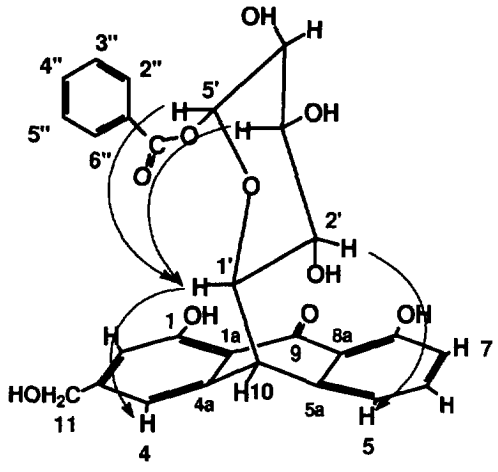

Fig. 1. ROESY effects shown by **1**.

and at *m/z* 256 for the aglycone. HRMS showed a $[M + 1]^+$ at *m/z* 447.1299 for compound **2**, and at $[M + 1]^+$ *m/z* 447.1302 for compound **3**, both corresponding to the formula $C_{22}H_{22}O_{10}$.

^{1}H NMR showed, in both cases, only two aromatic systems indicating a difference with respect to **1**; instead, both **2** and **3** showed a methyl group signal at δ1.7 for an acetyl group, suggesting that **2** and **3** are isomers and have an acetyl substituent at the 5′, instead of the benzoate substituent as in **1**. The ^{1}H NMR spectra of **2** and **3** were only differentiated in the aromatic pattern and the displacement of the signals; compound **2** showed, from δ6.86–7.07, a *doublet–singlet–doublet–singlet* pattern, while compound **3** showed from δ6.80–7.13, a *singlet–doublet–singlet–doublet* pattern.

^{13}C NMR showed little differences between these two compounds, suggesting that they are the C-10 isomers; this was apparent when both compounds, after a few hours in solution (DMSO-d_6) were interconvertible. HMBC NMR experiments, for both compounds, showed as important features, a correlation of the signal at δ1.7 (methyl) with the carbon at δ167 for the ester carbonyl group; the signal at δ4.6 for the proton on C-10 correlated to the signal for the protons on C-1′, C-1a, C-4, C-4a, C-5, C-5a and C-8a. Furthermore, ROESY NMR experiments, as in **1**, indicated the same sugar conformation, as previously reported for aloins and cascarosides [11, 12]. The NOE effect showed by the proton on C-1′ and C-2′ helps to differentiate between the *R* and *S* isomers; compound **2** showed an interaction between the proton on C-1′ (δ3.64–3.67) and the doublet at δ6.99 for the proton on C-5, whereas the proton on C-2′ (δ3.34–3.41) interacted with the singlet at δ7.07 for the proton on C-4, indicating that the absolute configuration of the C-10 in **2** is *S*. On the other hand, compound **3** showed, in the ROESY NMR experiment, a correlation between the signal at δ3.67–3.70 (C-1′) and the singlet at δ6.98 for the proton on C-4, whereas, the signal proton at δ3.38–3.44 (C-2′) correlated to the doublet at δ7.13 for the proton on C-5, indicating an *R* configuration at C-10 in compound **3**. The circular dichroism spectrum of **2** showed a positive Cotton effect at 294.60 nm and negative Cotton effects at 321.60 and 266.00 nm, as reported for (10*S*) aloin [11]. Similarly compound **3** showed a negative Cotton effect at 294.00 and positive Cotton effects at 317.00 and 268.80 nm as reported for (10*R*) aloin [11]. This establishes the absolute configuration of **2** as (10*S*) picramnioside B and of **3** as (10*R*) picramnioside C.

Aloe-emodin was identified for its spectroscopic properties (UV, HREIMS, ^{1}H and ^{13}C NMR) and by comparison of the ^{1}H NMR with a commercial sample (Apin Chemical, U.K.). Aloe-emodinanthrone was characterized using EIMS, ^{1}H and ^{13}C NMR and by comparison of previously reported values [14].

EXPERIMENTAL

General. The ^{1}H NMR spectra were recorded at 400 MHz and the ^{13}C NMR at 100 MHz, in the indicated solvent with TMS as int. standard. EIMS were obtained on a VG Analytical Instrument ZABSE mass spectrometer 8 eV.

Plant material. This was collected in Panama, province of Cocle in May 1990 and it was identified by Prof. Mireya Correa at the herbarium of the University of Panama, where a voucher specimen is deposited [Florpan 331 (PMA)].

Extraction. Dried and powdered leaves (600 g) of *P. antidesma* ssp. *fessonia* were extracted with MeOH by percolation. The extract was filtered and concd to a gum (127 g) under vacuum, and partitioned between H_2O and $CHCl_3$, the aq. layer was subsequently extracted with BuOH. The butanolic fr. (59 g) was submitted to CC (silica gel 60, 0.063–0.2 μm, Merck) using a glass column (4.5 × 75 cm) and $CHCl_3$, $CHCl_3$–MeOH (9:1, 8:2, 7:3) as eluent. Sixty frs (~ 125 ml) were obtained, frs 7–10 were washed with $CHCl_3$ and crystallized from $CHCl_3$ yielding 0.83 g of aloe-emodin; frs 18–27 were submitted to CC using $CHCl_3$, $CHCl_3$–MeOH (95:5, 9:1, 8:2) as eluent, yielding, after crystallization from $CHCl_3$–MeOH (9:1), 63 mg of **1**; frs 28–30 were submitted to CC using the same eluent as for **1**, yielding, after precipitation from MeOH–$CHCl_3$ (9:1), 0.43 g of amorphous powder of **2**; frs 31–35 were washed with MeOH and a residue soluble only in DMSO was obtained, which after repeated precipitations from DMSO by addition of $CHCl_3$ yielded 0.490 g of **3**. Frs 36–41 after TLC using $CHCl_3$–EtOAc (1:1) yielded 2 mg of aloe-emodin anthrone.

Oxidative hydrolysis. Compounds **2** (10 mg) and **3** (10 mg) were separately dissolved in 25 ml of 4 N HCl containing 1 mg of $FeCl_3$, and heated at 100° for 4 hr. The cooled soln was extracted with $CHCl_3$; the chloroformic fraction was submitted to PTLC using $CHCl_3$–MeOH (9:1) as solvent system. Each of the isolated compounds was identical to aloe-emodin on TLC ($CHCl_3$–MeOH 9:1) and their ^{1}H NMR spectra were superimposable.

Biological assays. The brine shrimp microwell assay was performed as previously described by Solis *et al.* [15] and the KB cell assay was performed as previously reported [3].

Picramnioside A (**1**). UV λ_{max}^{MeOH} nm: 221, 273, 302, 382, IR ν max (KBr) cm^{-1}: 3440 (OH), 2950, 1725, 1640, 1620, 1600, 1300. FDMS *m/z* (rel. int. %): 531 (100) $[M]^+$ + Na, 508 (12) $[M]^+$, 409 (18) $[M]^+$ − benzoate + Na, 122 (7) benzoate. FAB (glycerol/thioglycerol/TFA matrix (*m/z* (rel. int. %) 509 (17), 387 (54), 256 (46), 239 (26), 207 (40), 181 (53) 165 (37), 149 (34), 131 (34). HRMS (FAB) [M − benzoate]$^+$ *m/z* calcd 387.0716 ($C_{20}H_{19}O_8$) found 387.0724. CD (MeOH, c 86.5 μM) λ nm ($\Delta\varepsilon$): 342.8 (+ 1.68), 314.2 (− 0.027), 295.0 (− 6.36), 239.0 (+ 1.70), 230.6 (− 3.89), 221.2 (+ 14.4), ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.45–3.53 (*m*, 1H, H-2′), 3.67 (*m*, 2H, H-3′ and H-4′), 3.85 (*dd*, $J_{1'-10} = 2.1$, $J_{1'-2'} = 9.9$ 1H, H-1′), 4.03 (*d*, 2H, H-11), 4.65 (*d*, 1H, H-10), 4.91 (*d*, 1H, H-3′OH), 5.01 (*d*, 1H, 4′OH), 5.20 (*t*, 1H, H-11OH), 5.42 (*d*, 1H, H-2′OH), 5.62 (*s*, 1H, H-5′), 6.62 (*s*, 1H, H-2), 6.83 (*s*, 1H, H-4), 6.93 (*d*, 1H, H-7), 7.15 (*d*, 1H, H-5), 7.50–7.53 (*dd*, 2H, H-3″), 7.57–7.61 (*dd*, 1H, H-6), 7.65–7.69 (*dd*, 1H, H-4″), 7.71–7.73 (*dd*, 2H, H-2″), 11.83 (*s*, 1H, H-1), 11.96 (*s*, 1H, H-8). HMBC NMR experiment: proton (carbons) 2 (1a, 11), 3 (2, 3), 4 (1a, 2, 4a, 10, 11), 5 (6, 7, 8a, 10), 6 (5a), 7 (5, 6), 10 (1a, 4, 4a, 5a, 8a, 1′), 1′ (4a), 5′ (1′, 3′, 5′ C = O), 2″ (3″, 4″,5′C = O), 3″ (1″).

Picramnioside B (**2**). UV λ_{max}^{MeOH} nm: 225, 260, 270, 300, 365. UV λ max (MeOH + NaOH) 230, 265, 375, 390, 455 (sh). IR ν (KBr) cm^{-1}: 3350 (OH), 2730, 1760, 1640, 1620, 1600, 1590, 1295, 1230. FAB (glycerol/thioglycerol/TFA matrix) *m/z* (rel. int. %) 447 (48), 387 (16), 351 (46), 256 (100), 239 (39). HRMS (FAB) *m/z* calcd 447.1291 ($C_{22}H_{22}O_{10}$ + 1) found 447.1299; [M − acetyl] *m/z* calcd 387.0716 ($C_{20}H_{19}O_8$) found 387.0722. CD (MeOH, c 180 μM) λ nm ($\Delta\varepsilon$): 355.0 (+ 0.27), 336.2 (− 0.02), 321.6 (− 0.58), 308.6 (− 0.004), 294.6 (+ 1.25), 272.0 (− 0.003), 266.0 (− 0.94), 240.4 (+ 0.11), 226.6 (− 2.27), 210.8 (+ 2.43). ^{1}H NMR (DMSO d_6, 400 *m/z* MHz) δ 1.17 (*s*, 3H, H-5′ Me), 3.34–3.41 (*m*, 1H, H-2′), 3.46–3.48 (*m*, 2H, H-3′ and H-4′), 3.64–3.67 (*dd*, $J_{1'-10} = 2.17$, $J_{1'-2'} = 9.7$, 1H, H-1′), 4.58–4.62 (*m*, 3H, H-11, and H-10), 4.85 (*d*, 1H, H-3′, OH), 4.94 (*d*, 1H, H-4′OH), 5.32 (*d*, 1H, H-2′OH), 5.42 (*s*, 1H, H-5′), 5.46 (*t*, 1H, H-11, OH), 6.86 (*d*, 1H, H-7), 6.89 (*s*, 1H, H-2), 6.99 (*d*, 1H, H-5), 7.07 (*s*, 1H, H-4), 7.53–7.57 (*dd*, 1H, H-6), 11.85 (*s*, 1H, H-1, OH), 11.89 (*s*, 1H, H-8, OH). COSY 45, HMQC, HMBC and ROESY were used to confirm this assignment.

Picramnioside C (**3**). UV λ_{max}^{MeOH} nm: 225, 261 (sh), 270, 300, 365. IR ν (KBr) $cm,^{-1}$: 3500, 3390, 2990, 2920, 1725, 1640, 1620, 1600, 1300, 1250. FAB (glycerol/thioglycerol/TFA matrix) *m/z* (rel. int. %) 447 (63), 387 (48), 351 (26), 256 (100), 239 (40). HRMS *m/z* calcd 447.1291 ($C_{22}H_{22}O_{10}$ + 1) found 447.1302; [M − acetyl] *m/z* calcd 387.0716 ($C_{20}H_{19}O_8$) found 387.0712. CD (MeOH, *c*85 μM) UV λ nm ($\Delta\varepsilon$): 351.4 (+ 0.78), 317.0 (− 0.019), 294.0 (− 5.37), 268.8 (+ 0.009), 253.0 (+ 1.1), 230.0 (− 3.13), 210.6 (+ 6.47). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.72 (*s*, 3H, H-5′Me), 3.38–3.44 (*m*, 1H, H-2′), 3.54–3.57 (*m*, 2H, H-3′ and H-4′), 3.67–3.70 (*dd*, $J_{1'-10} = 2.0$, $J_{1'-2'} = 9.85$, 1H, H-1′), 4.51–4.63 (*m*, 3H, H-10 and H-11), 4.85 (*d*, 1H, H-3′ OH), 4.93 (*d*, 1H, H-4′ OH), 5.38 (*d*, 1H, H-2′ OH), 5.40 (*s*, 1H, H-5′), 5.48 (*t*, 1H, H-11 OH), 6.80 (*s*, 1H, H-2), 6.91 (*d*, 1H, H-7), 6.98 (*s*, 1H, H-4), 7.13 (*d*, 1H, H-5), 7.55–7.59 (*dd*, 1H, H-6), 11.84 (*s*, 1H, H-1 OH), 11.93 (*s*, 1H, H-8 OH). HMBC NMR experiment: proton (carbons) 1 OH (1, 2), 2 (1, 1a, 4, 11), 4 (2, 1a, 10, 11), 5 (10), 6 (8, 5a), 7 (5, 6, 8, 8a), 8 OH (8, 8a) 10 (1, 1a, 4a, 5a, 8a,1′), 11 (2, 3), 1′ (2′), 2′ (3′), 5′ (1′, 3′), 5′Me (5′ C = O).

Acknowledgements—We thank the E.E.C. Projects (B/CI1*-913064 and CI#1.050ESCJR) and Programa Regional de Desarrollo Cientifico y Tecnologico de la O.E.A. Also, we are indebted to Mrs Carmen Galdames for her valuable help in collection and plant preparation. We thank Dr J. Trujillo for running the CD spectra, Instituto Universitario de Bioorganica, Antonio Gonzalez, Tenerife, Spain. We are also indebted to Mrs J. Hawkes for running the NMR experiments in the AMX400 NMR, University of London Intercollegiate Research Service at King's College and Dr K. Welham for running the FAB experiments at the Mass Spectrometry Unit at The School of Pharmacy, University of London.

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