Pergamon

0031-9422(95)00765-2

Phytochemistry, Vol. 41, No. 4, pp. 1191–1195, 1996

0031-9422/96 $15.00 + 0.00

ANTIBACTERIAL HYDROPEROXYSTEROLS FROM *XANTHOSOMA ROBUSTUM**

TAKESHI KATO,† BARBARA FREI, MICHAEL HEINRICH‡ and OTTO STICHER

Department of Pharmacy, Swiss Federal Institute of Technology (ETH) Zurich, Winterthurerstrasse 190, CH-8057, Zürich, Switzerland; ‡ Institute of Pharmaceutical Biology, Schaenzlestrasse 1, D-79104 Freiburg, Germany

(*Received in revised form* 3 *July* 1995)

Key Word Index—*Xanthosoma robustum*; Araceae; hydroperoxides; antibacterial activity; 24-hydroperoxy-4α,14α-dimethylcholesta-8,26-dien-3β-ol; 25-hydroperoxy-4α,14α-dimethylcholesta-8,23-dien-3β-ol; 25-hydroperoxycycloart-23-en-3β-ol; 24-hydroperoxycycloart-25-en-3β-ol; 4α,14α-dimethylcholesta-8,24-dien-3β-ol; cycloartenol.

Abstract—Three new hydroperoxysterols, 24-hydroperoxy-4α,14α-dimethylcholesta-8,25-dien-3β-ol, 25-hydroperoxy-4α,14α-dimethylcholesta-8,23-dien-3β-ol and 25-hydroperoxycycloart-23-en-3β-ol, have been isolated from the aerial parts of *Xanthosoma robustum*, besides 24-hydroperoxycycloart-25-en-3β-ol, 4α, 14α-dimethylcholesta-8,24-dien-3β-ol and cycloartenol. Additionally, the two new diols, 4α,14α-dimethylcholesta-8,25-dien-3β,24-diol and 4α,14α-dimethylcholesta-8,23-dien-3β,25-diol were obtained from the first two hydroperoxysterols, respectively, by reduction with triphenylphosphine. The structures have been defined by chemical and spectroscopic studies. The four hydroperoxysterols exhibited antibacterial acitivities against *Escherichia coli*, *Bacillus subtilis* and *Micrococcus luteus*.

INTRODUCTION

Xanthosoma robustum Schott occurs in southern Mexico and Central America. The roots are used medicinally (external for wounds, skin diseases and culture bound syndromes) by the Indians of Oaxaca in Mexico, and the young leaves are cooked and eaten in Guatemala [1–3]. Chemically, the genus *Xanthosoma* is poorly known. In our detailed survey of the aerial parts, three new hydroperoxysterols, **1**–**3** have been isolated as well as 24-hydroperoxycycloart-25-en-3β-ol (**4**), 4α,14α-dimethylcholesta-8,24-dien-3β-ol (**5**) and cycloartenol (**6**). Additionally, the two new diols, **7** and **8** were obtained from **1** and **2**, respectively, by reduction with triphenylphosphine. In this paper, we report the isolation and structure elucidation of these compounds as well as their antibacterial activities.

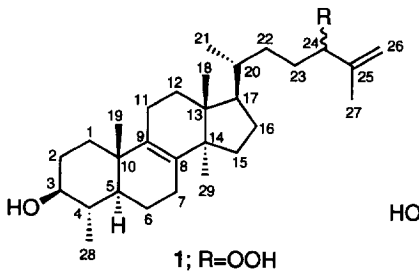
RESULTS AND DISCUSSSION

All compounds (**1**–**6**) were obtained from *n*-hexane extract of air-dried powdered aerial parts by a combination of VLC and HPLC as described in the Experimental. Identifications of **5** [4] and **6** [5] were achieved by comparison with previously reported spectroscopic data.

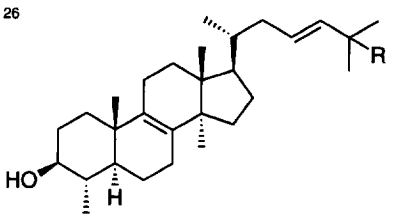
In the ^{13}C NMR spectrum, compound **1** showed 29 carbon signals which were similar to those of compound **5**. Since the chemical shifts of C-1 to C-21, C-28 and C-29 agreed very well with those of compound **5**, as shown in Table 1, compound **1** was suggested as a 4α,14α-dimethylcholesta-8-en-3β-ol derivative. The identification of the side chain in **1** was performed by analysis of the NMR spectra as follows. The methyl signal (H_3-27) at δ_H 1.73 and the two signals for protons (H_2-26) at δ_H 5.01 and 5.03 of vinyl carbon C-26 (δ_C 114.2) showed coupling to each other (J = 1.2 and 2.2 Hz, respectively), indicating that the isopropenyl group must be located at C-23. The carbon with a resonance at δ_C 90.4 (*d*) was confirmed at C-24, because H-24 was observed as triplet-like signal (J = 6.2 Hz) due to coupling with H-23 (see Experimental). In addition, in the ^{1}H–^{1}H COSY spectrum, H-24 showed a cross peak to H-26 due to its allyl coupling. This evidence supported the structure of the side chain as in **1**. The attachment of a hydroperoxy group at C-24 (δ_C 90.4) was indicated by the molecular ion $[M]^+$ at m/z 444 in the EI-mass spectrum. However, the carbons of C-24 to C-26 showed doubling of the signals (Table 1). This phenomenon is consistent with the presence of a mixture of C-24 epimers in compound **1** [6, 7]. Additionally, reduction of **1** with triphenylphosphine was attempted and yielded diol **7** as expected. The EI-mass spectrum of **7** gave $[M]^+$ at m/z 428. The ^{13}C NMR spectrum of **7** also showed signal doublings of C-23 to C-27. The resonances at δ_C 31.5, 76.3, 147.5, 111.4 and 17.2 (C-23 to C-27) were attributed to the 24*S* form, and the other resonances to the 24*R* form (Table 1) [8]. The signal of C-24 at δ_C 76.8 (or 76.3) was shifted upfield compared with that of **1** (δ_C 90.4 or 90.3). Similarly, in the ^{1}H NMR

*Dedicated to Prof. Dr H. Rimpler on the occasion of his 60th birthday.

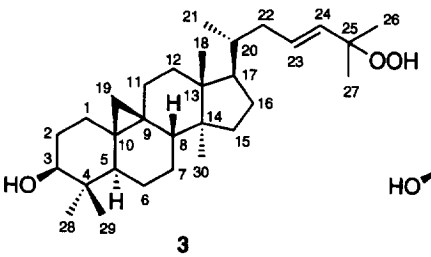
†Present address: Faculty of Pharmaceutical Sciences, Josai University, 1-1 Keyakidai, Sakado, Saitama, 350-02 Japan.



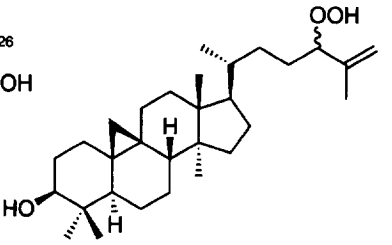
1; R=OOH
7; R=OH



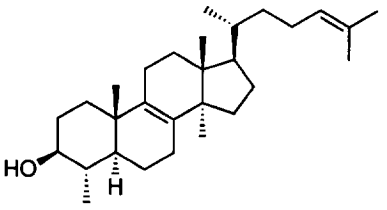
2; R=OOH
8; R=OH



3



4



5

spectrum, H-24 (δ_H 4.01) was shifted upfield in comparison with that of **1** (δ_H 4.27). These findings prove that compound **1** is a 24*S* and 24*R* mixture of 24-hydroperoxy-4α,14α-dimethylcholesta-8,25-dien-3β-ol.

In the EI-mass spectrum, compound **2** gave the same $[M]^+$ at *m/z* 444 as **1**. In the ^{13}C NMR spectrum, **2** also showed 29 carbon signals which were in agreement with those of **1** except for the signals for C-22 to C-27. These data suggest **2** to be isomer of **1**. The ^1H NMR spectrum of **2** showed two equivalent methyl groups as a singlet (6H) at δ_H 1.34 indicating H_3-26 and 27. The doublet (J_{24-23} = 16.0 Hz) and the doublet of doublet of doublets (J_{23-24} = 16.0, J_{23-22a} = 8.0 and J_{23-22b} = 6.0 Hz) due to one proton each at δ_H 5.52 and 5.69 were attributed to H-24 and H-23, respectively (Experimental). The carbon signal at δ_C 82.3 (*s*) was assigned to C-25 with a hydroperoxyl substitution. As expected, reduction of **2** with triphenylphosphine gave the corresponding diol **8** showing $[M]^+$ at *m/z* 428 in the EI-mass spectrum. In the ^{13}C NMR spectrum of **8**, the signal of C-25 (δ_C 70.7) was shifted upfield compared with that of **2** (δ_C 82.3), and the other carbon signals agreed very well with those of **2** except for the signals of two methyl groups (C-26 and C-27) bonding to C-25 and olefinic carbons (C-23 and C-24). The structure of **2** was thus deduced as 25-hydroperoxy-4α,14α-dimethylcholesta-8,23-dien-3β-ol.

Compound **3** gave $[M]^+$ at *m/z* 458 in the EI-mass spectrum and 30 carbon signals in the ^{13}C NMR spectrum. The carbon signals of C-1 to C-21 and C-28 to C-30 agreed with those of **6**, while those of C-22 to C-27 for the side chain were in good agreement with those of **2** (Table 1). In the ^1H NMR spectrum of **3**, two doublets (J = 4.0 Hz) at δ_H 0.33 and 0.55 were assigned to geminal methylene protons of a cyclopropane ring as in **6**. The olefinic proton signals (δ_H 5.69 and 5.52) and a singlet (δ_H 1.34, 6H) were very similar to those for H-23, H-24, H-26 and H-27 of **2** (Experimental). The structure of compound **3** was therefore established as 25-hydroperoxycycloart-23-en-3β-ol.

Compound **4** also exhibited $[M]^+$ at *m/z* 458 in the EI-mass spectrum. In the ^1H NMR spectrum of **4**, the geminal methylene protons of a cyclopropane ring were shown as two doublets (J = 4.0 Hz) at δ_H 0.33 and 0.55. A triplet (δ_H 4.27), signals for two olefinic protons (δ_H 5.01 and 5.03) and methyl group (δ_H 1.73) were very similar to those for H-24, H-26 and H-27 of **1** (Experimental). In the ^{13}C NMR data, C-1 to C-21 and C-28 to C-30 of 30 carbon signals in **4** agreed with those of **3**, and C-22 to

Table 1. ^{13}C NMR spectral data of compounds **1–8*** (δ, 75 MHz, $CDCl_3$)

C†	1		2	3	4		5	6	7		8
1	35.0		35.0	32.0	32.0		35.0	32.0	35.0		35.0
2	31.0		31.0	30.4	30.4		31.1	30.4	31.1		31.0
3	76.6		76.6	78.8	78.8		76.6	78.8	76.6		76.6
4	39.2		39.2	40.5	40.5		39.2	40.5	39.2		39.2
5	47.1		47.1	47.1	47.1		47.1	47.1	47.1		47.1
6	20.7		20.7	21.1	21.1		20.7	21.1	20.7		20.7
7	28.1		28.1	26.0	26.0		28.2	26.0	28.2		28.1
8	134.6		134.7	48.0	48.0		134.7	48.0	134.6		134.6
9	133.6		133.6	20.0	20.0		133.6	20.0	133.6		133.7
10	36.3		36.3	26.1	26.1		36.3	26.1	36.3		36.3
11	21.7		21.7	26.4	26.4		21.8	26.5	21.8		21.7
12	25.5		25.6	32.8	32.9		25.5	32.9	25.5		25.6
13	44.5		44.5	45.3	45.3		44.5	45.3	44.5		44.5
14	49.9		49.9	48.8	48.8		49.9	48.8	49.9		49.9
15	31.2		31.2	35.6	35.5		31.2	35.6	31.2		31.2
16	30.8		30.8	28.1	28.1		30.8	28.1	30.8		30.8
17	50.3		50.2	52.1	52.1	52.0§	50.4	52.3	50.3		50.2
18	15.7		15.8	18.1	18.0		15.7	18.0	15.7		15.8
19	18.2		18.2	29.9	29.9		18.2	29.9	18.7		18.2
20	36.2		36.6	36.3	36.0	35.8§	36.3	35.9	36.3		36.7
21	18.6		18.7	18.4	18.2	18.1§	18.6	18.2	18.2		18.7
22	27.2		39.4	39.4	27.6	27.3§	36.4	36.4	31.9		39.1
23	32.0		130.7	130.8	32.0		24.9	24.9	31.7	31.5§	125.6
24	90.4	90.3§	134.5	134.4	90.4	90.2§	125.3	125.3	76.8	76.3§	139.4
25	143.9	143.6§	82.3	82.3	143.9	143.6§	130.9	130.9	147.8	147.5§	70.7
26	114.2	114.7§	24.4‡	24.4‡	114.2	114.7§	17.6	17.6	110.9	111.4§	29.9‡
27	16.9		24.3‡	24.3‡	16.9	17.2§	25.7	25.7	17.6	17.2§	30.0‡
28	15.0		15.1	25.4	25.4		15.0	25.4	15.1		15.1
29	24.4		24.4	14.0	14.0		24.4	14.0	24.4		24.4
30				19.3	19.3			19.3			

*Multiplicities determined by DEPT sequences.
†Numbering according to the IUPAC-IUB (1989) recommendations.
‡Assignments in any vertical column may be interchanged.
§Signals for C-24 epimer.

C-27 for side chain were in good agreement with those of **1**. However, the ^{13}C NMR spectrum of **4** also showed doubling of certain carbons, i.e. C-17, C-20 to C-22 and C-24 to C-27 (Table 1). Based on this evidence, compound **4** was determined to be a 24-epimeric mixture of 24-hydroperoxycycloart-25-en-3β-ol. This compound (without assigning a precise stereochemistry at C-24) was isolated recently from *Euphorbia cyparissias* by Öksüz *et al.* [9]. For compound **4** complete NMR data are reported in Table 1 and in the Experimental, as they were found to be either incompletely reported or incorrectly assigned in some cases [9].

Compounds **1–4** showed significant antibacterial activities against *Escherichia coli*, *Bacillus subtilis* and *Micrococcus luteus* in contrast to **5–8**. Minimum growth inhibition concentrations on TLC are given in Table 2. These results suggest that the activities of **1–4** may be due to the hydroperoxy group.

Table 2. Antibacterial activities of **1–8** in comparison to chloramphenicol*

Compound	*E. coli*	*M. luteus*	*B. subtilis*
1	2	1	1
2	1	0.5	0.5
3	2	2	1
4	1	1	5
5	> 20	> 20	> 40
6	> 20	> 20	> 40
7	> 20	> 20	> 20
8	> 20	> 20	> 20
Chloramphenicol	0.04	0.04	0.04

*Minimum growth inhibitory amount in μg on TLC-plates.

EXPERIMENTAL

General. All mps are uncorr. Optical rotations were obtained on a Perkin-Elmer model 141 polarimeter. EI-MS were recorded on Hitachi-Perkin-Elmer-RMUGM mass spectrometer at 70 eV. All NMR spectra were measured with a Bruker AMX-300 spectrometer (300.13 MHz for ^1H and 75.47 MHz for ^{13}C) in $CDCl_3$. The residual $CHCl_3$ resonances at δ_H 7.26 and δ_C 77.0 were used as internal references. VLC was performed on Si gel

(type 60, Merck, 40–63 mm). HPLC separations were carried out on a LiChrosorb Si 60 column with a Waters model 590 pump and a Knauer differential refractometer.

Plant material. The plant material was collected near St. Domingo Petapa, Oaxaca, Mexico, in February 1993 and September 1994. Voucher specimens have been deposited at the following herbaria: MEXU (UNAM, México D.F.), ZT (ETH Zurich, Switzerland) and FB (Inst. Pharm. Biol., University of Freiburg, BRD).

Antibacterial activity. The pure compounds were assayed for antibacterial activity against *B. subtilis* (ATCC 6633), *E. coli* (ATCC 25922) and *M. luteus* (ATCC 9341), using a bioautographic method [10, 11].

Extraction and isolation. Air-dried and powdered plant material (1 kg) was extracted with *n*-hexane by percolation at room temp. to afford a crude extract (35 g), after solvent removal *in vacuo*. This material was subjected to VLC with hexane containing increasing portions of EtOAc to afford frs 1–10. Frs 1–3 (18.8 g) formed an oily mass containing lipids. Fr. 5 (1.9 g) gave β-sitosterol (*ca.* 150 mg) as white precipitate from CH_2Cl_2–MeOH. Frs 6–10 (8.7 g) formed a dark green mass containing chlorophylls. Fr. 4 (4.1 g) was fractionated by repeated VLC (×4) with hexane–EtOAc to give the following four frs of sterols, A (54 mg), B (36 mg), C (120 mg) and D (130 mg). The final purifications of these frs were carried out by HPLC. Frs A and B gave **1** (11 mg) and **2** (14 mg) with hexane–EtOAc (73:27) and **3** (8 mg) and **4** (13 mg) with hexane–EtOAc (4:1), respectively. Compounds **5** (37 mg) and **6** (21 mg) were obtained from frs C and D, respectively, with hexane–EtOAc (83:17).

24-*Hydroperoxy*-4α,14α-*dimethylcholesta*-8,25-*dien*-3β-*ol* (**1**). Amorphous powder. $[\alpha]_D^{25}$ + 54° ($CHCl_3$; *c* 0.2). EI-MS *m/z*: 444 $[M]^+$. ^{1}H NMR: δ0.70 (3H, *s*, H-18), 0.87 (3H, *s*, H-29), 0.90 (3H, *d*, *J* = 6.2 Hz, H-21), 0.96 (3H, *s*, H-19), 0.99 (3H, *d*, *J* = 6.8 Hz, H-28), 1.73 (3H, *dd*, *J* = 2.2, 1.2 Hz, H-27), 3.10 (1H, *ddd*, *J* = 9.8, 5.2 Hz, H-3), 4.27 (1H, *t*, *J* = 6.2 Hz, H-24), 5.01 (1H, *q*, *J* = 1.2 Hz, H-26a), 5.03 (1H, *q*, *J* = 2.2 Hz, H-26b), 7.74 (1H, *s*, OOH). ^{13}C NMR (Table 1).

25-*Hydroperoxy*-4α,14α-*dimethylcholesta*-8,23-*dien*-3β-*ol* (**2**). Amorphous powder. $[\alpha]_D^{25}$ + 54° ($CHCl_3$; *c* 0.2). EI-MS *m/z*: 444 $[M]^+$. ^{1}H NMR: δ 0.72 (3H, *s*, H-18), 0.87 (3H, *s*, H-29), 0.90 (3H, *d*, *J* = 6.2 Hz, H-21), 0.97 (3H, *s*, H-19), 0.99 (3H, *d*, *J* = 6.4 Hz, H-28), 1.34 (6H, *s*, H-26 and H-27), 3.10 (1H, *ddd*, *J* = 9.8, 5.2 Hz, H-3), 5.52 (1H, *d*, J_{24-23} = 16.0 Hz, H-24), 5.69 (1H, *ddd*, J_{23-24} = 16.0, J_{23-22a} = 8.0 and J_{23-22b} = 6.0 Hz, H-23), 7.24 (1H, *s*, OOH). ^{13}C NMR (Table 1).

25-*Hydroperoxycycloart*-23-*en*-3β-*ol* (**3**). Needles: mp 138–139°. $[\alpha]_D^{25}$ + 30° ($CHCl_3$; *c* 0.3). EI-MS *m/z*: 458 $[M]^+$. ^{1}H NMR: δ0.33 (1H, *d*, *J* = 4.0 Hz, 19-Ha), 0.55 (1H, *d*, *J* = 4.0 Hz, 19-Hb), 0.81 (3H, *s*, H-29), 0.87 (3H, *d*, *J* = 6.2 Hz, H-21), 0.88 (3H, *s*, H-30), 0.965 (3H, *s*, H-28)*, 0.970 (3H, *s*, H-18)*, 1.34 (6H, *s*, H-26 and H-27), 3.28 (1H, *m*, $W_{1/2}$ = 13.0 Hz, H-3), 5.52 (1H, *d*, *J* = 16.0 Hz, H-24), 5.69 (1H, *ddd*, *J* = 16.0, 8.0, 6.0 Hz, H-23), 7.23 (1H, *s*, OOH), *assignments may be exchangeable. ^{13}C NMR (Table 1).

24-*Hydroperoxycycloart*-25-*en*-3β-*ol* (**4**). Needles: mp 128–129°. $[\alpha]_D^{25}$ + 46° ($CHCl_3$; *c* 0.4). EI-MS *m/z*: 444 $[M]^+$. ^{1}H NMR: δ 0.33 (1H, *d*, *J* = 4.0 Hz, 19-Ha), 0.55 (1H, *d*, *J* = 4.0 Hz, 19-Hb), 0.81 (3H, *s*, H-29), 0.87 (3H, *d*, *J* = 7.8 Hz, H-21), 0.89 (3H, *s*, H-30), 0.96 (3H, *s*, H-28)*, 0.97 (3H, *s*, H-18)*, 1.73 (3H, *dd*, *J* = 2.2, 1.2 Hz, H-27), 3.28 (1H, *m*, $W_{1/2}$ = 15.0 Hz, H-3), 4.27 (1H, *t*, *J* = 6.2 Hz, H-24), 5.01 (1H, *br s*, H-26a), 5.03 (1H, *m*, $W_{1/2}$ = 5.0 Hz, H-26b), 7.73 (1H, *s*, OOH), *assignments may be exchangeable. ^{13}C NMR (Table 1).

Reduction of hydroperoxysterols. The soln (2 ml) of **1** (5 mg) in CH_2Cl_2 was treated with triphenylphosphine (excess) at room temp. for 1 hr. After removal of the excess triphenylphosphine and triphenylphosphine oxide by CC, the purification of the reduction product by HPLC with hexane–EtOAc (4:1) yielded diol **7** (3.6 mg). Using the same conditions **2** gave the corresponding diol **8**.

4α,14α-*Dimethylcholesta*-8,25-*dien*-3β,24-*diol* (**7**). Needles: mp 175–176°. EI-MS *m/z*: 428 $[M]^+$. ^{1}H NMR: δ 0.71 (3H, *s*, H-18), 0.87 (3H, *s*, H-29), 0.91 (3H, *d*, *J* = 6.2 Hz, H-21), 0.97 (3H, *s*, H-19), 0.99 (3H, *d*, *J* = 6.8 Hz, H-28), 1.72 (3H, *br s*, H-27), 3.10 (1H, *ddd*, *J* = 9.8, 5.2 Hz, H-3), 4.01 (1H, *m*, $W_{1/2}$ = 10.0 Hz, H-24), 4.83 (1H, *q*, *J* = 1.8 Hz, H-26a), 4.93 (1H, *m*, $W_{1/2}$ = 5.2 Hz, H-26b). ^{13}C NMR (Table 1).

4α,14α-*Dimethylcholesta*-8,23-*dien*-3β,25-*diol* (**8**). Needles: mp 222–223°. EI-MS *m/z*: 428 $[M]^+$. ^{1}H NMR: δ 0.71 (3H, *s*, H-18), 0.87 (3H, *s*, H-29), 0.89 (3H, *d*, *J* = 6.2 Hz, H-21), 0.97 (3H, *s*, H-19), 0.99 (3H, *d*, *J* = 6.4 Hz, H-28), 1.32 (6H, *s*, H-26 and H-27), 3.10 (1H, *m*, $W_{1/2}$ = 15.0 Hz, H-3), 5.59 (2H, *m*, $W_{1/2}$ = 4.0 Hz, H-23 and H-24). ^{13}C NMR (Table 1).

Acknowledgements—We thank Mr F. Ramos, Herbario Nacional (MEXU) for identification of plant material. We are also extremely grateful to Dr A. D. Wright, ETH Zurich, for measuring NMR and for very helpful comments, Mr O. Greter, ETH Zurich, for recording all mass spectra, and Dr E. Zass, ETH Zurich, for performing literature searches.

REFERENCES

1. Plowman, T. (1969) *Econ. Bot.* **23**, 116.
2. Frei, B., Sticher, O. and Heinrich, M. (1994) *Medicinal Plants of the Lowland Zapotecs (Oaxaca, Mexico): Multidisciplinary Evaluation of an Indigenous Pharmacopoeia*, Botanikertagung '94, Bayreuth, Germany, Abstract No. P 14.08.
3. Heinrich, M. (1989) Ethnobotanik der Tieflandmixe (Oaxaca, Mexico) und phytochemische Untersuchung von *Capraria biflora* L. (Scrophulariaceae). Dissertationes Botanicae No. 144. Berlin und Stuttgart. J. Cramer.
4. Harref, A. B. and Lavergne, J.-P. (1985) *Bull. Soc. Chim. Fr.* 965.

5. Kamisako, W., Honda, C., Suwa, K. and Isoi, K. (1987) *Magn. Reson. Chem.* **25**, 683.
6. Anjaneyulu, V., Rao, G. S. and Connolly, J. D. (1985) *Phytochemistry* **24**, 1610.
7. Anjaneyulu, V., Prasad, K. H., Ravi, K. and Connolly, J. D. (1985) *Phytochemistry* **24**, 2359.
8. Greca, M. D., Fiorentino, A., Monaco, P. and Previtera, L. (1994) *Phytochemistry* **35**, 1017.
9. Öksüz, S., Gil, R. R., Chai, H., Pezzuto, J. M., Cordell, G. A. and Ulubelen, A. (1994) *Planta Med.* **60**, 594.
10. Baumgartner, B., Erdelmeier, C. A. J., Wright, A. D., Rali, T. and Sticher, O. (1990) *Phytochemistry* **29**, 3327.
11. Hamburger, M. O. and Cordell, G. A. (1987) *J. Nat. Prod.* **50**, 19.