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ANTIBACTERIAL TRITERPENOID ACIDS
FROM *DILLENIA PAPUANA*

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ABSTRACT.—Three new oleanene-type triterpenoids, dillenic acids A [**1**], B [**2**], and C [**3**], and two known compounds, 3-oxoolean-1,12-dien-30-oic acid [**4**] (a new natural product) and the lupene derivative betulinaldehyde, have been isolated from the Papua New Guinean medicinal plant *Dillenia papuana*. The structures of the new compounds were elucidated as 2α-hydroxy-3-oxoolean-12-en-30-oic acid, 2-oxo-3β-hydroxyolean-12-en-30-oic acid and 1α-hydroxy-3-oxoolean-12-en-30-oic acid. The ^{1}H- and ^{13}C-nmr data of all new compounds were assigned unambiguously using a variety of 2D nmr experiments including ^{1}H-^{1}H-COSY, HMQC, HMBC, and NOESY experiments. Of these compounds, **1–4** showed antibacterial activities against *Bacillus subtilis*, *Escherichia coli*, and *Micrococcus luteus*.

Dillenia papuana Martelli in Becc. (Dilleniaceae) is a tree, the bark of which is used in the traditional medicine of Papua New Guinea for the treatment of asthma and severe chest pains and to assist in child delivery (1,2). Earlier workers on the genus *Dillenia* reported the isolation of triterpenoid compounds (3) and flavonoids (4,5). In a preliminary biological screening, the petroleum ether extract of *D. papuana* showed antibacterial activity. Biological activity-guided fractionation yielded four oleanene-type triterpenoids, dillenic acids A [**1**], B [**2**], and C [**3**] as new metabolites and the known 3-oxoolean-1,12-dien-30-oic acid [**4**] (a new natural product). Together with these compounds, the known lupene derivative betulinaldehyde was also isolated. In this paper the isolation and structure elucidation of these compounds, as well as their antibacterial activities, are reported.

RESULTS AND DISCUSSION

Air-dried and powdered leaves and some stems (2.8 kg) of *Dillenia papuana*, collected near Lae in the Morobe Province of Papua New Guinea, were extracted with petroleum ether by percolation at room temperature to afford, after solvent removal, a crude extract. Fractionation of this material by a combination of vlc and mplc, followed by hplc, as described in the Experimental, led to the isolation of five triterpenoid compounds.

The molecular formula of compound **1** was determined as $C_{30}H_{46}O_4$ (m/z 470.3350) from its accurate mass measurement, indicating it to be a triterpene having eight degrees of unsaturation. Ir absorptions at 3400, 1720, and 1700 cm^{-1} and ^{13}C-nmr resonances at δ 182.4 (s), 69.2 (d), and 216.6 (s) indicated the presence of a carboxylic acid group, a secondary hydroxyl, and a ketone functionality in **1**. Seven singlet resonances for methyl groups (δ_H 0.81, 1.01, 1.10, 1.12, 1.16, 1.19, and 1.28) and a broad triplet at δ_H 5.31 were observed in the ^{1}H-nmr spectrum (Table 1). This, together with the ^{13}C-nmr resonances at δ 122.3 (d) and 144.4 (s) (Table 2), suggested the presence of a $\Delta^{12,13}$ double bond of an oleanene system (6). The mass spectrum revealed a pair of diagnostically important fragment peaks at m/z 248 (base peak) and m/z 203 (m/z 248−CO$_2$H), which are typical for a retro-Diels-Alder fragmentation in ring C of olean-12-ene derivatives containing a carboxyl function in either ring D or E (7,8). The presence of the
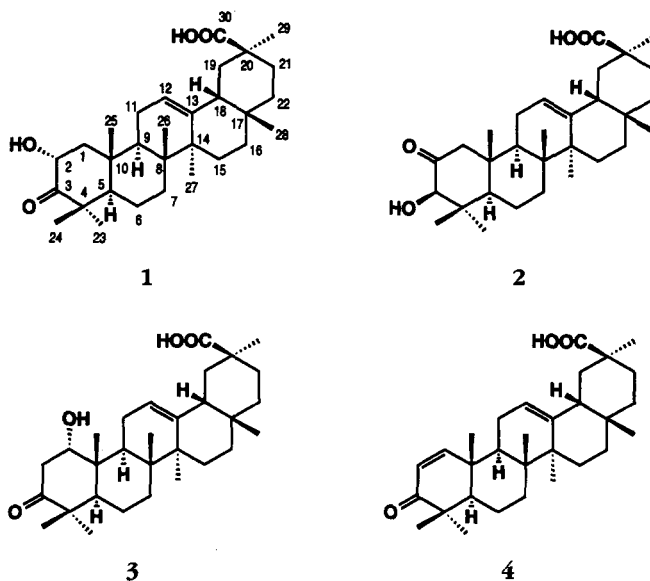
oleanene skeleton was further verified using nmr spectroscopy, in particular an HMBC measurement. Using this procedure, all carbons with the exception of C-2, C-6, C-11, and C-12 could be associated with one or more of the methyl groups on the basis of $^2J_{CH}$ and $^3J_{CH}$ correlations. The carbon with a resonance at δ_C 19.2 was confirmed at C-6, because H_2-6 showed a cross-peak to H-5 in the ^1H-^1H-COSY spectrum. The carboxylic acid group was assigned to C-20 on the basis of an HMBC correlation to H_3-29.

A comparison of the ^{13}C-nmr data for C-12, C-13, C-18–C-21, and C-28–C-30 with those of several 18α/18β-olean-12-en-20α/20β-oic acid derivatives (9,10) and koetjapic acid (11) revealed the geometry of the D/E ring junction (cis, 18β-H) and suggested the C-20 carboxylic acid group to be β and axially oriented. The partial structure of the segment C-1–C-3 was elucidated by a combination of HMBC and ^1H-^1H-COSY experiments. The protons of the methylene carbon (δ_C 49.6) and H-2 showed coupling to each other ($J = 12.6$ Hz), indicating that the ketone carbonyl must reside at position C-1 or C-3. Long-range correlations between H_3-23, H_3-24, and the ketone carbonyl group enabled it to be assigned to C-3. Because the signal at δ_C 49.6 of the methylene carbon showed long-range correlation to H_3-25 and because H_2-1 coupled to H-2, the hydroxyl group was assigned to C-2. The stereochemistry of the hydroxyl group was elucidated by NOESY nmr experiments and analysis of ^1H-^1H coupling constants. Diagnostic nOes were observed between H_{ax}-2/H_3-25, H_{ax}-2/H_3-24, H_3-24/H_3-25, and H_3-25/H_3-26. These data, in addition to the coupling constant between H-2 and H_{ax}-1 ($J = 12.6$ Hz) supported the α and equatorial configuration of the hydroxyl group. On this evidence, compound 1 was identified as the new natural product 2α-hydroxy-3-oxoolean-12-en-30-oic acid, for which the trivial name dillenic acid A is proposed.

A comparison of the ^{13}C-nmr data of compounds 2–4 with those of compound 1 suggested a close similarity between these four molecules. The only differences between them were located in ring A. Compound 2 was shown to have the same molecular formula as compound 1, $C_{30}H_{46}O_4$, by accurate mass measurement. In its ir spectrum, absorbances for the same functional groups as in 1 were observed. The reduction of the two double doublets in 1 (δ 2.42 and 4.56) to two doublets (δ 2.07 and 2.49) in the ^1H-nmr spectrum indicated that the methylene protons of the carbon with a resonance at δ_C 53.4 and the proton of the hydroxyl group-bearing carbon were no longer vicinal but

TABLE 1.   ¹H-Nmr Data[a] (CDCl₃, 300.13 MHz) of Triterpenes 1–4.

Proton	Compound			
	1	2	3	4
H-1α	2.42	2.07	—	—
H-1β	1.12[b]	(d, J=12.2 Hz)		
	(dd, J=6.7 and 12.6 Hz)	2.49	3.97	—
		(d, J=12.2 Hz)	(dd, J=3.0 and 3.6 Hz)	
H-1	—	—	—	7.05
				(d, J=10.1 Hz)
H-2α	—	—	2.37	—
			(dd, J=3.0 and 16.2 Hz)	
H-2β	4.56	—	3.06	—
	(dd, J=6.5 and 12.6 Hz)		(dd, J=3.6 and 16.2 Hz)	
H-2	—	—	—	5.81
				(d, J=10.1 Hz)
H-3α	—	3.91 s	—	—
H-5	1.21[b]	1.48[b]	1.75[b]	1.58[b]
H-6	1.55[b]	1.48[b], 1.70[b]	1.57[b]	1.58[b]
H-7	1.39[b], 1.55[b]	1.42[b], 1.64[b]	1.40[b], 1.60[b]	1.45[b], 1.60[b]
H-9	1.63[b]	1.89[b]	2.42[b]	1.88[b]
H-11	1.97[b]	1.88[b]	1.96[b]	2.12[b]
H-12	5.31 br t	5.30 br t	5.31 br t	5.37 br t
H-15	1.01[b], 1.79[b]	1.04[b], 1.81[b]	1.01[b], 1.83[b]	1.02[b], 1.84[b]
H-16	0.92[b], 1.93[b]	0.92[b], 1.96[b]	0.91[b], 1.93[b]	0.92[b], 1.96[b]
H-18	1.99[b]	2.01[b]	2.00[b]	2.03[b]
H-19	1.60[b], 1.86[b]	1.63[b], 1.86[b]	1.62[b], 1.88[b]	1.67[b], 1.88[b]
H-21	1.37[b], 1.93[b]	1.38[b], 1.97[b]	1.35[b], 1.92[b]	1.39[b], 1.96[b]
H-22	1.36[b]	1.37[b]	1.36[b]	1.38[b]
H-23	1.16 s	1.20 s	1.11 s	1.16 s
H-24	1.12 s	0.70 s	1.07 s	1.10 s
H-25	1.28 s	0.90 s	1.16 s	1.19 s
H-26	1.01 s	0.96 s	1.05 s	1.06 s
H-27	1.10 s	1.19 s	1.18 s	1.16 s
H-28	0.81 s	0.81 s	0.82 s	0.83 s
H-29	1.19 s	1.20 s	1.18 s	1.21 s

[a]δ in ppm.
[b]¹H-Nmr chemical shifts assigned on the basis of a ¹³C-¹H COSY experiment. Signal multiplicity was not assigned due to signal overlap.

probably separated by the ketone. The position of the hydroxyl group was proposed as C-3, β and equatorial on the basis of nOe cross-peaks observed between H_{ax}-3/H_{ax}-1, H_{ax}-3/H-5, and H_{ax}-3/H_3-23. Dillenic acid B [2] was thus established as 2-oxo-3β-hydroxyolean-12-en-30-oic acid.

The accurate mass measurement of compound 3 showed its molecular formula to be the same as that of compounds 1 and 2, $C_{30}H_{46}O_4$. Its ir absorptions at 3490 and 1700 cm⁻¹ and ¹³C-nmr resonances at δ 73.7 (d), 181.5 (s), and 215.8 (s) indicated the presence of a secondary hydroxyl, a carboxylic acid, and a saturated ketone function. The ketone carbonyl was assigned to C-3 on the basis of HMBC correlations between H_{ax}-2/C-3, H_{eq}-2/C-3, H_{eq}-1/C-3, H_3-23/C-3, and H_3-24/C-3. The oxygen-bearing carbon with resonance at $δ_C$ 73.7 showed a long-range correlation to H_3-25, while its proton showed coupling to the methylene protons of the carbon with a resonance at $δ_C$ 43.0, indicating them to be vicinal. These correlations clearly suggested the hydroxyl group to be located at C-1. A NOESY correlation between H-1 and H_3-25 showed that the hydroxyl group was α-oriented. The axial position of the OH group was supported by the coupling constants between H_{eq}-1 and the two protons at C-2 (dd, J=3.6 and 3.0 Hz). Therefore, 3 was identified as 1α-hydroxy-3-oxoolean-12-en-30-oic acid, dillenic acid C.

Compound 4 was analyzed for $C_{30}H_{44}O_3$ (*m/z* 452.3284) by mass spectrometry. From its ir spectrum the presence of a carboxyl group (3400, 1700 cm⁻¹) and an α,β-unsaturated keto group (1670 cm⁻¹), which was supported by its uv spectrum [λ max (MeOH) nm (log ε) 229 (3.96)], were indicated. ¹H- and ¹³C-nmr comparison of the

TABLE 2. ^{13}C-Nmr Data[a] (CDCl$_3$, 75.47 MHz) of Triterpenes **1–4**.

Carbon	Compound			
	1	**2**	**3**	**4**
C-1	49.6 t[b]	53.4 t	73.7 d	159.1 d
C-2	69.2 d	211.3 s	43.0 t	125.0 d
C-3	216.6 s	83.1 d	215.8 s	205.3 s
C-4	47.7 s	44.3 s	47.8 s	44.6 s
C-5	57.7 d	54.7 d	47.7 d	53.4 d
C-6	19.2 t	18.8 t	19.2 t	18.9 t
C-7	32.3 t	32.5 t	31.9 t	32.5 t
C-8	39.9 s	40.4 s	39.7 s	40.6 s
C-9	47.3 d	47.8 d	37.5 d	41.8 d
C-10	37.6 s	45.9 s	41.0 s	41.9 s
C-11	23.7 t	23.6 t	23.2 t	23.5 t
C-12	122.3 d	122.2 d	122.2 d	122.1 d
C-13	144.4 s	144.7 s	144.7 s	144.8 s
C-14	41.5 s	41.9 s	42.1 s	39.4 s
C-15	26.1 t	26.3 t	26.2 t	26.1 t
C-16	26.9 t	27.1 t	27.0 t	26.9 t
C-17	31.9 s	32.1 s	32.0 s	32.0 s
C-18	47.9 d	48.2 d	48.0 d	48.2 d
C-19	42.5 t	42.7 t	42.7 t	42.4 t
C-20	44.0 s	43.8 s	44.0 s	44.0 s
C-21	31.1 t	31.2 t	31.1 t	31.1 t
C-22	38.2 t	38.4 t	38.3 t	38.2 t
C-23	24.7 q	29.6 q	25.3 q	27.8 q
C-24	21.6 q	16.8 q	21.9 q	21.7 q
C-25	16.2 q	16.6 q	15.6 q	18.8 q
C-26	17.0 q	16.6 q	17.0 q	17.3 q
C-27	26.0 q	26.1 q	26.0 q	25.9 q
C-28	28.1 q	28.3 q	28.2 q	28.2 q
C-29	28.7 q	28.9 q	28.6 q	28.7 q
C-30	182.4 s	183.3 s	181.5 s	182.1 s

[a]δ in ppm.
[b]Multiplicities determined by DEPT sequences.

chemical shifts for C-1, C-2, and C-3 [δ$_C$ 159.1, 125.0, and 205.3 and δ$_H$ at 7.05 (d, J=10.1 Hz) and 5.81 (d, J=10.1 Hz)] with those of glomeric acid (12) indicated this latter moiety to be located between C-4 and C-10. Further support for this deduction came from the COSY spectrum (cross-peak between H-1 and H-2) and an HMBC measurement which showed correlations between H-1/C-3, H-1/C-5, H-1/C-10 and H-2/C-4. Thus, compound **4** is the new natural product 3-oxoolean-1,12-dien-30-oic acid. This compound has been obtained previously by derivatization of glycyrrhetic acid (13).

Together with these new compounds a known metabolite was isolated and its structure confirmed to be identical with that of betulinaldehyde.

Compounds **1–4** showed antibacterial activities against *B. subtilis, E. coli,* and *M. luteus* (Table 3). It is suspected that the carboxylic group and the Δ^{12,13} double bond play an important role in determining the observed activity. A study by Fried *et al.* (14), concerning antibacterial steroid and triterpenoid acids showed that, aside from the polycyclic skeleton, the occurrence of an oxygen function or a double bond in the γ- or δ-position with respect to a carboxylic group appears to be necessary for antibacterial activity. In this context, it is important to point to the possible phytoalexin role of triterpenoid acids to protect the plant against microbial attacks (15,16).

TABLE 3.   Antibacterial Activities of Triterpenes **1**–**4**.

Compound	Minimum growth inhibition amount in μg on tlc		
	B. subtilis	*E. coli*	*M. luteus*
1	2.4	2.4	1.2
2	2.0	1.0	1.0
3	2.0	5.0	2.0
4	1.0	1.0	1.0
Chloramphenicol	0.1	0.04	0.04

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Optical rotations were obtained on a Perkin-Elmer model 141 polarimeter. Ir and uv spectra were recorded on a Perkin-Elmer 781 infrared spectrometer and a Kontron Uvikon 930 spectrometer, respectively. Eims were recorded on a Hitachi-Perkin-Elmer-RMUGM mass spectrometer at 70 eV. All nmr spectra (^{1}H-, ^{13}C, COSY, NOESY, HMQC, and HMBC) were recorded on a Bruker AMX-300 spectrometer (300.13 MHz for ^{1}H 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 μm). Mplc was effected on Si gel (type 60$_{HF254}$, Merck, 15 μm), using a Büchi 681 pump and Büchi mplc columns (800 mm×36 mm i.d. and 800 mm×49 mm i.d.). Hplc separations were carried out on a LiChrosorb Si 60 column (250 mm×16 mm i.d., 5 μm) from Knauer with a Waters model 590 pump and a Knauer differential refractometer.

PLANT MATERIAL.—The aerial parts of *D. papuana* were collected in April 1991, near Lae in the Morobe Province, Papua New Guinea. A voucher specimen has been deposited at the Rijksherbarium, University of Leiden, the Netherlands.

BIOASSAYS.—The crude extract, chromatographic fractions, and pure compounds were assayed for antibacterial activity against *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, and *Micrococcus luteus* ATCC 9341, using a bioautographic method (17).

EXTRACTION AND ISOLATION.—A 2.8-kg quantity of air-dried and powdered plant material was extracted with petroleum ether by percolation at room temperature. Solvent was removed *in vacuo* to obtain a crude extract (90.1 g, 3.2%). This material was divided into six parts and subjected to vlc with hexane containing increasing portions of EtOAc to afford 16 fractions of 200 ml each. Tlc control led to the reduction of these to 9 major fractions, which were tested for antibacterial activity. Fractions 4–9 showed antibacterial properties. Fraction 7 (1350 mg), obtained from vlc with hexane-EtOAc (25:75), was subjected to mplc with hexane-CH$_2$Cl$_2$-HCOOH (80:20:2) and afforded 195 fractions, 10 ml each. Based on tlc and ^{1}H-nmr measurements, fractions 35–59 were chosen for final purification by hplc [hexane-CH$_2$Cl$_2$-EtOAc-HCOOH (46:46:8:2)], and yielded compounds **1** (97 mg) and **2** (46 mg). Combined vlc fractions 8 and 9 (1500 mg) were also further fractionated by mplc. Separation of fractions 83–98 was achieved on hplc and afforded compounds **3** (15 mg) and **4** (5 mg). Both separations were carried out with the same eluent used for the purification of compounds **1** and **2**. Mplc separation of vlc fraction 3 (500 mg) with hexane-CH$_2$Cl$_2$-EtOAc (8:1:1) yielded 215 fractions, 10 ml each. Hplc purification of fractions 30–36 with hexane-Me$_2$CO (9:1) gave betulinaldehyde (10 mg).

Dillenic acid A [**1**].—A white amorphous powder: [α]^{25}D +177.2° (*c*=0.2, CHCl$_3$); ir ν max (film) 3400, 2980–2870, 1720, 1700, 1460, 1380, 1220, 1100 cm^{-1}; ^{1}H nmr, see Table 1; ^{13}C nmr, see Table 2; HMBC correlations (CDCl$_3$, H/C) 1$_{ax}$/2, 1$_{eq}$/2, 2$_{ax}$/4, 12/9, 12/11, 13/27, 23/3, 23/4, 23/5, 23/24, 24/3, 24/4, 24/5, 24/23, 25/1, 25/5, 25/9, 25/10, 26/7, 26/8, 26/9, 26/14, 27/8, 27/13, 27/14, 27/15, 28/16, 28/17, 28/18, 28/22, 29/19, 29/20, 29/21, 29/30; NOESY correlations (CDCl$_3$, H/H) 1$_{eq}$/25, 1$_{eq}$/2$_{ax}$, 1$_{eq}$/11$_{eq}$, 2$_{ax}$/24, 2$_{ax}$/25, 24/25, 25/26; eims *m/z* [M]$^{+}$ 470 (2), 455 (2), 424 (2), 314 (2), 249 (18), 248 (100), 203 (6); hreims *m/z* [M]$^{+}$ 470.3350 (calcd for C$_{30}$H$_{46}$O$_4$, 470.3398).

Dillenic acid B [**2**].—A colorless amorphous powder: [α]^{25}D +71.3° (*c*=0.48, CHCl$_3$); ir ν max (film) 3490, 3020–2800, 1710, 1460, 1390, 1380, 1260, 1230, 1150, 1060 cm^{-1}; ^{1}H nmr, see Table 1; ^{13}C nmr, see Table 2; NOESY correlations (CDCl$_3$, H/H) 1$_{eq}$/25, 3$_{ax}$/1$_{ax}$, 3$_{ax}$/23, 3$_{ax}$/5, 24/25, 25/26; eims *m/z* [M]$^{+}$ 470 (3), 455 (2), 424 (1), 409 (1), 261 (1), 259 (1), 250 (2), 249 (18), 248 (100), 203 (5); hreims *m/z* [M]$^{+}$ 470.3383 (calcd for C$_{30}$H$_{46}$O$_4$, 470.3398).

Dillenic acid C [**3**].—A white amorphous powder: [α]^{25}D +107.6° (*c*=0.06, CHCl$_3$); ir ν max (film)

3490, 2980–2820, 1700, 1460, 1380 cm^{-1}; ^{1}H nmr, see Table 1; ^{13}C nmr, see Table 2; HMBC correlations (CDCl$_3$, H/C) 1$_{eq}$/3, 1$_{eq}$/5, 1$_{eq}$/10, 1$_{eq}$/25, 2$_{ax}$/1, 2$_{ax}$/3, 2$_{eq}$/1, 2$_{eq}$/3, 23/3, 23/4, 23/5, 23/24, 24/3, 24/4, 24/5, 24/23, 25/1, 25/5, 25/9, 25/10, 26/7, 26/8, 26/9, 26/14, 27/8, 27/13, 27/14, 27/15, 28/16, 28/17, 28/18, 28/22, 29/19, 29/20, 29/21, 29/30; NOESY correlations (CDCl$_3$, H/H) 1$_{eq}$/25, 2$_{ax}$/24, 2$_{ax}$/25; eims m/z [M]$^+$ 470 (<1), 452 (8), 437 (2), 408 (1), 406 (2), 250 (2), 249 (18), 248 (100), 203 (7); hreims m/z [M−H$_2$O]$^+$ 452.3312 (calcd for C$_{30}$H$_{44}$O$_3$, 452.3291).

3-Oxooolean-1,12-dien-30-oic acid [**4**].—A colorless amorphous powder: [α]^{25}D +113.6° (c=0.12, CHCl$_3$); ir ν max (film) 3400, 2980–2850, 1700, 1670, 1460, 1380, 1260, 1230, 1160, 825 cm^{-1}; uv λ max (MeOH) nm (log ε) 229 (3.96); ^{1}H nmr, see Table 1; ^{13}C nmr, see Table 2; HMBC correlations (CDCl$_3$, H/C) 1/3, 1/5, 1/10, 2/4, 2/10, 23/4, 23/5, 23/24, 24/4, 24/5, 24/23, 25/5, 25/9, 25/10, 26/7, 26/8, 26/9, 26/14, 27/8, 27/14, 27/15, 28/16, 28/17, 28/18, 28/22, 29/19, 29/20, 29/21; eims m/z [M]$^+$ 452 (7), 437 (4), 406 (2), 391 (1), 315 (1), 287 (1), 250 (2), 249 (18), 248 (100), 203 (4); hreims m/z [M]$^+$ 452.3284 (calcd for C$_{30}$H$_{44}$O$_3$, 452.3291).

Betulinaldehyde [3β-*Hydroxy-20(29)-lupen-28-al*].—A colorless amorphous powder: [α]^{25}D +19.5° (c=0.1, CHCl$_3$) [lit. (18) [α]^{27}D +19.2° (c=1.1, CHCl$_3$)]; eims m/z [M]$^+$ 440 (38), 232 (17), 220 (15), 207 (59), 189 (86); ^{1}H- and ^{13}C-nmr data were identical with literature values (19,20).

ACKNOWLEDGMENTS

LITERATURE CITED

1. L. Balun and D. Holdsworth, *Int. J. Crude Drug Res.*, **26**, 51 (1988).
2. D. Holdsworth, *Int. J. Crude Drug Res.*, **25**, 231 (1987).
3. S. Dan and S.S. Dan, *J. Ind. Chem. Soc.*, **67**, 760 (1980).
4. S.D. Srivastava, *Phytochemistry*, **20**, 2445 (1981).
5. A.A. Gurni and K. Kubitzki, *Biochem. Syst. Ecol.*, **9**, 109 (1981).
6. J.D. Connolly and R.A. Hill, in: "Methods in Plant Biochemistry, Volume 7, Terpenoids," Ed. by B.V. Charlwood and D.V. Banthorpe, Academic Press, London, 1991, pp. 343–345.
7. H. Budzikiewicz, J.M. Wilson, and C. Djerassi, *J. Am. Chem. Soc.*, **85**, 3688 (1963).
8. L. Ogunkoya, *Phytochemistry*, **20**, 121 (1981).
9. H. Duddeck, M.H.A. Elgamal, G.S. Ricca, B. Danieli, and G. Palmisano, *Org. Magn. Reson.*, **11**, 130 (1978).
10. G.S. Ricca, B. Danieli, G. Palmisano, H. Duddeck, and M.H.A. Elgamal, *Org. Magn. Reson.*, **11**, 163 (1978).
11. N. Kaneda, J.M. Pezzuto, A.D. Kinghorn, N.R. Farnsworth, T. Santisuk, P. Tuchinda, J. Udchachon, and V. Reutrakul, *J. Nat. Prod.*, **55**, 654 (1992).
12. Y. Shiobara, S.S. Inoue, K. Kato, Y. Nishiguchi, Y. Oishi, N. Nishimoto, F. De Oliveira, G. Akisue, M.K. Akisue, and G. Hashimoto, *Phytochemistry*, **32**, 1527 (1993).
13. J.S. Baran, D.D. Langford, C.D. Liang, and B.S. Pitzele, *J. Med. Chem.*, **17**, 184 (1971).
14. J. Fried, G.W. Krakower, D. Rosenthal, and H. Basch, *J. Med. Chem.*, **8**, 279 (1965).
15. R. van der Heijden, E.R. Verheij, J. Schripsema, A. Baerheim Svendsen, R. Verpoorte, and P.A.A. Harkes, *Plant Cell Rep.*, **7**, 51 (1988).
16. T.K. Chen, D.C. Ales, N.C. Baenziger, and D.F. Wiemer, *J. Org. Chem.*, **48**, 3525 (1983).
17. J. Orjala, A.D. Wright, H. Behrends, G. Folkers, O. Sticher, H. Rüegger, and T. Rali, *J. Nat. Prod.*, **57**, 18 (1994).
18. J.D. Connolly and R.A. Hill, "Dictionary of Terpenoids," Chapman and Hall, London, 1991, Vol. 2, p. 1259.
19. K. Nakajima, H. Taguchi, T. Endo, and I. Yosioka, *Chem. Pharm. Bull.*, **26**, 3050 (1978).
20. P. Monaco and L. Previtera, *J. Nat. Prod.*, **47**, 673 (1984).