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Labdane diterpenoids from *Croton oblongifolius*

S. Roengsumran *, A. Petsom, D. Sommit, T. Vilaivan

Department of Chemistry, Faculty of Science, Chulalongkorn University, Phayathai Road, Pathumwan, Bangkok 10330, Thailand

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Abstract

Four new labdane diterpenoids, labda-7,12(*E*),14-triene, labda-7,12(*E*),14-triene-17-al, labda-7,12(*E*),14-triene-17-ol, labda-7,12(*E*),14-triene-17-oic acid, were isolated from the stem bark of *Croton oblongifolius*. The structure of these compounds were established by spectroscopic data and chemical transformation.

Keywords: Croton oblongifolius; Euphorbiaceae; Labdane; Diterpenoid

1. Introduction

Croton oblongifolius Roxb. (Euphorbiaceae) is widely distributed in Thailand. It has been used as a traditional medicine for many applications such as for dysmenorrhea, as a purgative and to treat dyspepsia and dysenteria. Even though *C. oblongifolius* has been intensively investigated by Seshadri et al. (Rao, Sachdev, Seshadri, & Singh, 1968; Aiyar & Seshadri, 1970, 1971, 1972a,b, we found that the same plant in Thailand contains different chemical constituents, possibly due to the geographic variation. Our recent investigation on this plant has led to the isolation of four new labdane diterpenoids, labda-7,12(*E*),14-triene (**1**), labda-7,12(*E*),14-triene-17-al (**2**), labda-7,12(*E*),14-triene-17-ol (**3**) and labda-7,12(*E*),14-triene-17-oic acid (**4**). In this report we present a full account of the structure elucidation of **1**, **2**, **3** and **4** by one- and two-dimensional NMR spectroscopy and by chemical transformation.

2. Results and discussion

The concentrated methanolic extract of the stem bark of *C. oblongifolius* was partitioned with hexane. The crude hexane extract was separated by chromatography on a silica gel column using a hexane-ethyl acetate gradient system to obtain four new labdane diterpenoids **1**–**4**. Treatment of **4** with diazomethane in ether gave **5**. Compound **5** was reduced with lithium aluminum hydride to produce a compound identical to **3**.

The molecular formula of compound **1** was assigned as $C_{20}H_{32}$ based on elemental analysis, ^{1}H and ^{13}C NMR (Table 1) and EIMS [M^{+}] (*m*/*z* 272). Its mass spectrum exhibited a molecular ion at *m*/*z* 272, corresponding to a bicyclic diterpene consisting of three double bonds. The ion of *m*/*z* 191 was formed by loss of a side chain. The DEPT NMR spectrum indicated the presence of five singlet methyl signals, six methylene carbons, two methine carbons and four quaternary carbons, two –C=CH– groups [δ 5.42 (1H-7, ddd, J = 1.5,1.5,4.0), 5.51 (1H-12, br.t, J = 6.7); δ 122.6 (C-7), 135.0 (C-8), 135.9 (C-12), 132.4(C-13)] and one –HC=CH_2 group [δ 6.35 (1H-14, dd, J = 10.7, 17.4), 4.89 (1H-15, d, J = 10.7), 5.04 (1H-15, d, J = 17.4); δ 141.7 (C-14) and 109.8 (C-15)]. Detailed analysis of 2-D ^{1}H and ^{13}C NMR spectrum, including COSY, NOESY, HMQC and HMBC supported a labdane-type structure. The stereochemistry of the $\Delta^{12,13}$ double bond was assigned as *E*, based on the cross peaks observed between H-12 and H-14 in the NOESY spectrum of Compound **1** (Fig. 1). Comparison of the spectroscopic data of compound **1** with that published for the known labda-7,12(*Z*),14-triene (Barrero, Sanchez, Alrarez-ManZaneda, Munoz, & Haidaur, 1992) indicated that compound **1** differs from the reported compound only at the stereochemistry of the

* Corresponding author.

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Table 1
^{1}H and ^{13}C NMR data (δ in ppm, J in Hz) for compounds **1** and **2** in $CDCl_3$

	Compound **1**		Compound **2**	
Position	δ_H	HMBC (H to C)	δ_H	HMBC (H to C)
1	1.00ddd (J = 3.4,3.4,13.4), 1.85m	C-3,C-2,C-20	1.00dd (J = 3.7,3.7,13.1), 1.90ddd (J = 3.0,5.2,13.1)	C-3,C-9
2	1.45m, 1.53m	C-3	1.40m, 1.50m,	C-1,C-5,C-9
3	1.16ddd (J = 3.7,3.7,13.1), 1.41m	C-1,C-2,C-4,C-10,C-18,C-19	1.16m, 1.43m	C-1,C-2,C-4,C-5
4	–		–	
5	1.19dd (J = 4.9,12.2)	C-4,C-6,C-9,C-10,C-18,C-19,C-20	1.13dd (J = 4.3,12.5)	C-4,C-6,C-7,C-9,C-10,C-19,C-20
6	1.87m, 1.97m	C-7,C-8,C-10	2.18m, 2.34m,	C-5,C-7,C-8,C-10
7	5.42ddd (J = 1.5,1.5,4.0)	C-5,C-6,C-9,C-17	6.83ddd (J = 2.1,2.1,5.8),	C-5,C-6,C-9,C-17
8	–		–	
9	1.89m	C-5,C-12	2.31m	C-5,C-8,C-10
10	–		–	
11	2.11ddd (J = 7.6,7.6,16.5) 2.29ddd (J = 1.8,4.0,16.5)	C-8,C-9,C-10,C-12,C-13,C-14	2.47ddd (J = 2.1,6.4,16.8), 2.62ddd (J = 7.0,7.0,16.8),	C-8,C-9,C-10,C-12,C-13,C-14
12	5.51br.t(J = 6.7)	C-9,C-11,C-14	5.44br.t(J = 6.7)	C-9,C-11,C-14,C-16
13	–		–	
14	6.35dd (J = 10.7,17.4)	C-12,C-13	6.29dd, (J = 10.9,17.4)	C-11,C-12,C-13,C-16
15	4.89d(J = 10.7), 5.04d(J = 17.4)	C-13,C-14,C-16	4.84d(J = 10.9), 5.00d(J = 17.4)	C-13,C-14
16	1.74s	C-13,C-14,C-15	1.70s	C-12,C-13,C-14
17	1.60s	C-7,C-8	9.37s	C-7,C-8,C-9
18	0.86s	C-3,C-4,C-5	0.86s	C-3,C-4,C-5,C-19
19	0.88s	C-3,C-4,C-5	0.91s	C-3,C-4,C-5
20	0.79s	C-1,C-9,C-10	0.79s	C-1,C-9,C-10,C-11

$\Delta^{12,13}$ double bond. Thus compound **1** was assigned as labda-7,12(*E*),14-triene (Fig. 1).

Compound **2** was isolated as colorless needle-like crystals. The molecular formula of $C_{20}H_{30}O$ was established by microanalysis and EIMS [M^+] (m/z 286). The IR spectrum showed a moderate intensity C–H stretching band for an aldehyde functional group at 2852 and 2710 cm^{-1} and a strong absorption band for an α,β-unsaturated carbonyl group of an aldehyde at 1690 cm^{-1}. The presence of the aldehyde function was supported by ^{13}C NMR spectrum, which showed a signal for C-17 at δ 194.3. The ^{1}H NMR spectrum of **2** showed a sharp singlet signal at δ 9.37 which was assigned to the aldehyde proton attached to C-17. This conclusion was supported by the long-range couplings observed in the HMBC spectrum (Table 1) which established the correlation between the aldehyde proton and C-7, C-8 and C-9 and the correlation of H-7 with C-17. Comparison of the spectral data (Table 1) of this compound with that of **1**, indicated that compound **2** differed from **1** only in having the aldehyde group at C-17 in place of the C-7 methyl group of compound **1**. The above data suggested that compound **2** was labda-7,12(*E*),14-triene-17-al (Fig. 1).

Compound **3** was isolated as colorless crystals. The molecular formula of $C_{20}H_{32}O$ was established by microanalysis and EIMS [M^+] (m/z 288). The ^{13}C NMR spectrum (Table 2) of **3** was similar to that of labda-7,12(*E*),14-triene (**1**) except for the downfield position of C-17 (δ 66.0), compared to that of **1** (δ

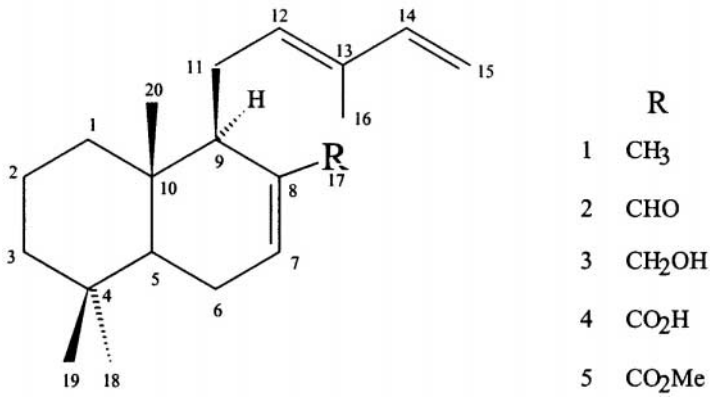

Fig. 1.

Table 2
^{1}H and ^{13}C NMR data (δ in ppm, J in Hz) for Compounds **3** and **4** in $CDCl_3$

	Compound **3**		Compound **4**	
Position	δ_H	HMBC (H to C)	δ_H	HMBC (H to C)
1	1.01ddd (J = 3.7,3.7,13.1), 1.89m	C-3,C-2,C-20	1.01ddd (J = 3.4,3.4,12.5), 1.87ddd (J = 3.7,5.2,12.5)	C-3,C-2,C-20
2	1.45m, 1.53m	C-1,C-3	1.41d(J = 2.5), 1.50m	C-3
3	1.16ddd (J = 3.7,3.7,13.4), 1.40m	C-1,C-2,C-4	1.15ddd (J = 3.1,3.1,14.0), 1.42m	C-1,C-2,C-4,C-10,C-18,C-19
4	–		–	
5	1.23dd (J = 4.9,12.5)	C-3,C-4,C-7,C-9, C-10,C-18,C-20	1.17dd(J = 4.3,12.2)	C-4,C-6,C-9,C-10,C-18,C-19,C-20
6	1.90m, 2.05m	C-7,C-8,C-10	2.03dddd (J = 2.1,4.3,12.2,19.2), 2.19dddd (J = 2.1,4.3,6.1,19.2),	C-7,C-8,C-10
7	5.75ddd (J = 2.5,2.5,5.2)	C-5,C-6,C-9,C-17	6.90ddd (J = 2.1,2.1,6.1)	C-5,C-6,C-9,C-17
8	–		–	
9	2.07m	C-5,C-7,C-12	2.37m	C-5,C-12
10	–		–	
11	2.33br.d (J = 16.5), 215ddd (J = 8.2,8.2,16.5)	C-8,C-9,C-12,C-13	2.30br.dd (J = 6.1,16.8), 2.58ddd (J = 7.3,7.3,16.8)	C-8,C-9,C-10,C-12,C-13,C-14
12	5.55br.t(J = 6.7)	C-9,C-11,C-14	5.47br.t(J = 6.4)	C-9,C-11,C-14
13	–		–	
14	6.34dd (J = 10.7,17.4)	C-12,C-13,C-16	6.31dd(J = 10.9,17.4)	C-12,C-13
15	4.91d(J = 10.7), 5.06d(J = 17.4)	C-13,C-14,	4.84d(J = 10.9), 5.00d(J = 17.4)	C-13,C-14,C-16
16	1.75s	C-12,C-13,C-14	1.67s	C-13,C-14,C-15
17	3.86d(J = 12.8), 4.05ddd (J = 0.9,1.8,12.8)	C-7,C-8	–	–
18	0.85s	C-3,C-4,C-5,C-19	0.86s	C-3,C-4,C-5
19	0.87s	C-3,C-4,C-5	0.89s	C-3,C-4,C-5
20	0.77s	C-1,C-5,C-9,C-10	0.82s	C-1,C-9,C-10

22.5). Its DEPT NMR spectrum indicated that C-17 was a methylene group corresponding to the two double doublet signals (δ 3.86, d, J = 12.8 and δ 4.05, ddd, J = 0.9,1.8,12.8) of H_2-17 in its ^{1}H NMR spectrum. The IR spectrum showed a broad absorption band for the hydroxy group at 3249 cm^{-1}. Comparison of the spectral data including ^{1}H NMR and ^{13}C NMR (DEPT, NOESY, HMQC and HMBC) of this compound with that of **1** demonstrated that compound **3** differed from **1** only in having a hydroxy group attached to C-17. Based on the above spectral data, the structure of **3** was assigned as labda-7,12(*E*),14-triene-17-ol (Fig. 1).

Compound **4** was isolated as colorless crystals. The molecular formula was proposed to be $C_{20}H_{30}O_2$, based on microanalysis and EIMS [M^+] (m/z 302). The IR spectrum showed a broad absorption band between 3421–2627 cm^{-1} and a strong absorption peak at 1707 cm^{-1}, characteristic of a carboxylic group. The presence of a carboxylic moiety was supported by a ^{13}C resonance at δ 174.1. The ^{13}C NMR spectral data (Table 2) were similar to those of **1**, **2** and **3**, except that the functional group of this compound at C-17 was a carboxylic acid. Thus, the structure of **4** was proposed to be labda-7,12(*E*),14-triene-17-oic acid (Fig. 1) and the assignment was further supported by analysis of its 2-D NMR spectra (Table 2).

The relative stereochemistries of compounds **1**–**4** were determined on the basis of NOESY spectra. Key NOE correlations in compound **1** are shown in Fig. 2. The relative stereochemistries of compounds **2**–**4** were similarly assigned and these were consistent with the published data of labdane-type structure (Urones, Marcos, Martin, Brito Palma, & Rodilla, 1987; Barrero et al., 1992; Zhou et al., 1997).

Compound **5**, a colorless oil, was the methyl ester of compound **4**, and was prepared by treatment of **4** with diazomethane in ether. The ^{13}C NMR spectrum of **5** was similar to that of compound **4** except for the signal of C-17 for the carbonyl carbon of a carboxylate ester (δ 169.5) which moved upfield compared to the carbonyl carbon of carboxylic acid in **4** (δ 174.1). When **5** was reduced with lithium aluminum hydride in dry ether followed by silica gel column chromatography eluting with ether/hexane, an alcohol was obtained. The ^{1}H and ^{13}C NMR spectra of this alcohol were identical with those of compound **3**.

3. Experimental

Mps uncorr.: on a Fisher–Johns melting point apparatus. Optical rotations: on a JASCO DIP-370 digital polarimeter. UV spectra: on a Hewlett Packard 8452A. IR spectra: on a Perkin Elmer 1760X FT–IR

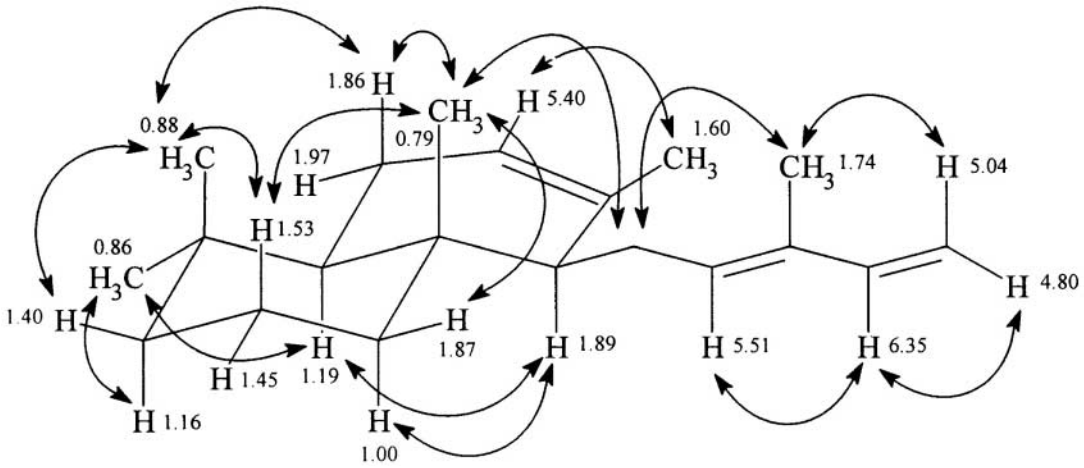

Fig. 2.

Spectrophotometer. Low-resolution MS: 70 eV, on a Fisons Instruments Trio 2000 mass spectrometer. ^{1}H and ^{13}C NMR, HMQC and HMBC experiments were recorded on a JEOL JNM-A500 spectrometer. Microanalyses were determined on a Perkin Elmer PE 2400 Series II.

3.1. Plant material

The stem bark of *C. oblongifolius* was collected from Ampur Pranburi, Prachuab Kirikhan Province, Thailand, in September 1996. Botanical identification was achieved through comparison with a voucher specimen No. BKF 084729 in the herbarium collection of the Royal Forest Department of Thailand.

3.2. Extraction and isolation

The powdered, sun-dried stem bark (2.5 kg) of *C. oblongifolius* was extracted with MeOH (5×5 l). The MeOH extract was filtered, and evaporated in vacuo to obtain a dark-red gummy residue which was re-extracted with hexane to give a hexane extract as a yellowish green oil (90 g). The hexane extract (40 g) was fractionated by silica gel CC and eluted with hexane–EtOAc into four fractions, I (4.52 g)(hexane), II (1.94 g)(5% EtOAc in hexane), III (4.65 g) and IV (25.50 g)(10% EtOAc in hexane). Compound **1** (4.25 g, 0.17%) was obtained by CC of fraction I on silica gel, eluting with hexane. Compound **2** (1.6 g, 0.06%), Compound **3** (4.29 g, 0.17%) and Compound **4** (23.64 g, 0.94%) were obtained in the same fashion from fractions II, III and IV, using EtOAc in hexane as eluant, respectively.

3.3. labda-7,12(E),14-triene (*1*)

Viscous transparent oil, (Found: C, 88.1; H, 11.6. $C_{20}H_{32}$ requires: C, 88.2; H, 11.8%), $[\alpha]_D^{30}$ +3.77° ($CHCl_3$; *c*1.76); UV λ_{max} EtOH nm (log ε): 232sh (4.44); IR ν_{max}^{neat} cm^{-1}: 2924, 2867, 1672, 1645, 1453, 1384; ^{1}H and ^{13}C NMR: Table 1. EIMS *m/z* (rel. int.): 272[M^+](4), 191(99), 135(14), 121(31), 109(100), 95(58), 81(29), 69(30), 55(35), 41(43).

3.4. labda-7,12(E),14-triene-17-al (*2*)

Colorless needle-crystals, mp 72–74°, (Found: C, 83.9; H, 10.7. $C_{20}H_{30}O$ requires: C, 83.9; H 10.5%); $[\alpha]_D^{30}$ +37.48° ($CHCl_3$; *c* 1.51); UV λ_{max} EtOH nm (log ε): 232 sh (4.53); IR ν_{max}^{KBr} cm^{-1}: 2954, 2923, 2852, 2710, 1690, 1639, 1607, 1460, 1443, 1384, 1368; ^{1}H and ^{13}C NMR: Table 1. EIMS *m/z* (rel. int.): 286[M^+](18), 271(15), 191(10), 163(14), 147(13), 124(24), 109(100), 91(38), 81(62), 55(22), 41(25).

3.5. labda-7,12(E),14-triene-17-ol (*3*)

Colorless needle-crystals, mp 90–92°, (Found: C, 83.2; H, 11.3. $C_{20}H_{30}O$ requires: C, 83.4; H 11.1%); $[\alpha]_D^{30}$ +12.02° ($CHCl_3$; *c*1.63); UV λ_{max} EtOH nm (log ε): 234 sh (4.54); IR ν_{max}^{KBr} cm^{-1}: 3249, 2919, 2845, 1645, 1640, 1607, 1459, 1441, 1388, 1365, 1073, 1057; ^{1}H and ^{13}C NMR: Table 2. EIMS *m/z* (rel. int.): 288[M^+](3), 270(19), 255(15), 202(12), 189(21), 176(20), 161(16), 147(25), 131(52), 109(100), 105(69), 91(84), 81(92), 55(76), 41(76).

3.6. labda-7,12(E),14-triene-17-oic acid (*4*)

Colorless needle-crystals, mp 118–120°, (Found: C, 79.5; H, 9.7. $C_{20}H_{30}O$ requires: C, 79.5; H 9.9%); $[\alpha]_D^{30}$ −15.93° ($CHCl_3$; *c* 1.67); UV λ_{max} EtOH nm (log ε): 232sh (4.47); IR ν_{max}^{KBr} cm^{-1}: 3421–2627, 2946, 1707, 1652, 1604, 1459, 1430, 1383, 1345, 1208; ^{1}H and ^{13}C NMR: Table 2. EIMS *m/z* (rel. int.): 302[M^+](50), 284(14), 221(47), 203(44), 175(65), 151(67), 139(100), 125(80), 109(95), 81(94), 69(68), 55(64), 41(67).

Table 3
^{13}C NMR spectral data of compound **1**–**5** (125 MHz,$CDCl_3$, ppm)

Position	1	2	3	4	5
1	39.7	39.9	39.6	40.1	39.9
2	18.9	18.5	18.8	18.6	18.6
3	42.3	41.9	42.2	41.9	42.0
4	33.0	32.9	33.0	32.8	33.3
5	50.2	49.4	49.8	49.3	50.3
6	23.8	24.9	23.6	24.0	23.7
7	122.6	151.8	125.4	140.5	137.0
8	135.0	143.4	138.6	133.6	134.5
9	55.2	49.4	52.2	50.0	50.3
10	36.8	36.7	36.7	36.9	36.8
11	26.3	24.8	25.9	26.0	26.2
12	135.9	133.8	134.8	133.4	133.6
13	132.4	132.7	133.5	133.1	133.1
14	141.7	141.8	141.3	141.8	141.7
15	109.8	109.8	110.7	109.9	110.0
16	11.9	11.8	11.9	11.7	11.7
17	22.5	194.3	66.0	174.1	169.5
18	33.3	33.2	33.2	33.3	32.8
19	22.0	22.1	22.0	22.2	22.1
20	14.0	14.7	14.0	14.8	14.5
CH_3O–					51.3

3.7. Methyl labda-7,12(E),14-triene-17-oate (5)

Ester **5** was prepared by methylation of **4** (100 mg, 0.33 mmol) with CH_2N_2 in Et_2O. The reaction product was purified by silica gel CC eluting with $CHCl_3$–hexane (1:1) to give **5** as a viscous oil (100.45 mg, 96%); $[\alpha]_D^{30}$ −5.64° ($CHCl_3$; *c* 1.26); UV λ_{max} EtOH nm (log ε): 230sh (4.17); IR ν_{max}^{neat} cm^{-1}: 2945, 2923, 1713, 1639, 1605, 1469, 1446, 1395, 1372, 1247, 1077; ^1H NMR (200 MHz, $CDCl_3$): δ 6.62(1H,dt), 6.30(1H,dd), 5.42(1H,t), 5.01(1H,d), 4.85(1H,d), 3.59(3H,s), 2.50(1H,m), 2.38(1H,m), 2.22(1H,m), 2.12(1H,dt), 2.08(1H,m), 1.87(1H,dd), 1.68(3H,s), 1.47(1H,m), 1.43(1H,m), 1.38(1H,m), 1.27(1H,d), 1.14(1H,d), 1.03(1H,dd), 0.88(3H,s), 0.85(3H,s), and 0.81(3H,s); ^{13}C NMR: Table 3. EIMS *m/z* (rel. int.): 316[M^+](46), 284(50), 235(42), 203(70), 175(96), 165(56), 153(80), 139(55), 119(67), 190(100), 105(71), 93(53), 91(75), 81(79), 79(68), 69(59), 55(68), 41(9).

3.8. Reduction of compound 5

A solution of **5** (80 mg, 0.25 mmol) in 10 ml of dry Et_2O was reduced with $LiAlH_4$ (62.37 mg, 1.64 mmol) for 5 h at room temperature. The product was purified by silica gel CC eluting with 30% Et_2O in hexane to give semisynthetic **3** (58.32 mg, 80%), identical in all respects (NMR, IR and MS) with the natural material.

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References

Aiyar, V. N., & Seshadri, T. R. (1970). *Tetrahedron, 26,* 5275.
Aiyar, V. N., & Seshadri, T. R. (1971). *Indian J. Chem., 9,* 1028.
Aiyar, V. N., & Seshadri, T. R. (1972a). *Phytochemistry, 11,* 1473.
Aiyar, V. N., & Seshadri, T. R. (1972b). *Curr. Sci., 41,* 839.
Barrero, A. F., Sanchez, J. F., Alrarez-ManZaneda, E. J., Munoz, M., & Haidaur, A. (1992). *Phytochemistry, 31,* 615.
Rao, P. S., Sachdev, G. P., Seshadri, T. R., & Singh, H. B. (1968). *Tetrahedron Lett*., 4685.
Urones, J. G., Marcos, I. S., Martin, D. D., Brito Palma, F. M. S., & Rodilla, J. M. (1987). *Phytochemistry, 26,* 3037.
Zhou, B. N., Baj, N. J., Glass, T. E., Malone, S., Werkhoven, M. C. M., Van Troon, F., David, Wisse, J. H., & Kingston, D. G. I. (1997). *J. Nat. Prod., 60,* 1287.