Plant Anticancer Agents, L. Cytotoxic Triterpenes from Sandoricum koetjape Stems

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PLANT ANTICANCER AGENTS, L.[1] CYTOTOXIC TRITERPENES FROM *SANDORICUM KOETJAPE* STEMS

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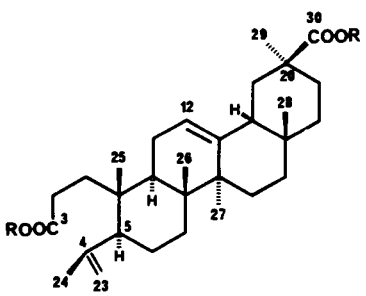
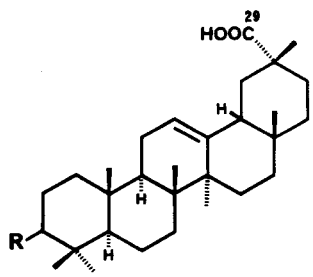
ABSTRACT.—A new ring-A secotriterpene, koetjapic acid [**1**], and five known compounds, 3-oxo-olean-12-en-29-oic acid [**2**] (a novel natural product), katonic acid [**3**], (−)-alloaromadendrene, (−)-caryophyllene oxide, and (+)-spathulenol, have been isolated and characterized from a cytotoxic Et_2O-soluble extract of *Sandoricum koetjape* stems. Of these compounds, **2** and **3** demonstrated significant cytotoxic activity against cultured P-388 cells (ED_{50} values of 0.61 and 0.11 μg/ml, respectively). Significant, albeit less intense, cytotoxicity was also observed with a variety of cultured human cancer cells. The ^{13}C-nmr chemical shifts of these triterpenes were assigned unambiguously using selective INEPT nmr experiments. Aside from compounds **2** and **3**, these substances were not toxic with cultured cells.

The stems of *Sandoricum koetjape* Merr. [syn. *Sandoricum indicum* (Cav.) Santol] (Meliaceae), a medium-sized tree (2), were collected in Thailand as part of our collaborative search for novel antineoplastic agents of plant origin (1). Decoctions of the bark are drunk after childbirth in Malaysia (3) and for the treatment of diarrhea in Thailand (4). Previous phytochemical investigations conducted with various parts of this species have demonstrated fatty acids in the seed oil (5), triterpenes from the heartwood (6) and fruit hulls (7), and limonoids from the seeds (8).

In this paper, we report details of the isolation, structure elucidation, and cytotoxic evaluation of a new ring-A secotriterpene, koetjapic acid [**1**], two known triterpenes, 3-oxo-olean-12-en-29-oic acid [**2**] (a new natural product) and katonic acid [**3**], and three known sesquiterpenes, (−)-alloaromadendrene, (−)-caryophyllene oxide, and (+)-spathulenol.



1 R=H



2 R=O
3 R=αOH

[1]For the previous paper in this series, see Kaneda *et al.* (1).

RESULTS AND DISCUSSION

The molecular formula of **1** was determined as $C_{30}H_{46}O_4$ (*m/z* 470.3398) from its high resolution mass spectrum, indicating that it was a triterpene. The presence of two carboxyl groups was inferred by a combination of ir spectral (ν max 1700 cm^{-1}) and ^{13}C-nmr spectral (δ 176.3 and 179.4) observations. In addition, signals representing six singlet methyls (δ 0.89, 0.95, 1.04, 1.27, 1.39, and 1.83) and an exo-methylene group (δ 4.89 and 4.98) were observed in the ^1H-nmr spectrum. Thus, compound **1** was considered to be a secotriterpene. The ^{13}C-nmr chemical shifts at δ 122.7 (d) and 145.0 (s) and the eims base peak at *m/z* 248 suggested that it might be a seco-olean-12-ene type compound and that one of the carboxyl groups was in rings C–E (C-27 through C-30). The position of this carboxyl group was determined as occurring at C-30 by a selective INEPT experiment, an nmr technique that identifies vicinal ^{13}C-^1H coupling (9). Thus, irradiation ($^3J_{CH}$=4 Hz) of the methyl proton at δ 1.39 (H-29), which correlated with δ 29.1 (q, C-29) (10) in the ^1H-^{13}C HETCOR spectrum of **1**, resulted in enhancements of carbons at δ 44.3 (s, C-20) and 179.4 (s, C-30). It is known that ring-A fissioned triterpenes are the most common secotriterpenes (11). From the chemical shift (δ 1.83) of the H-24 methyl protons in the ^1H-nmr spectrum of **1**, and by comparison of ^{13}C-nmr chemical shifts of its methyl ester derivatives with those of nyctanthic acid Me ester (12), it was confirmed that a second carboxyl group in **1** was at C-3 (δ 176.3) and that the exo-methylene group occurred at C-23 (δ 113.8, t). Therefore, this new ring-A secotriterpene, to which we have accorded the trivial name koetjapic acid [**1**], was elucidated as 3,4-seco-olean-4(23),12-diene-3,30-dioic acid.

Compound **2** exhibited seven singlet methyl protons (δ 0.87, 1.02, 1.06, 1.07, 1.10, 1.15, and 1.23) in the ^1H-nmr spectrum and 30 carbons, including peaks at δ 122.5 (d), 143.9 (s), 185.3 (s) and 217.8 (s), in the ^{13}C-nmr spectrum, suggesting that it was an oxo-olean-12-enoic acid. It was also speculated that the position of a carbonyl group (δ 217.8) was at C-3, because no oxygen-attached carbon peaks were observed in the δ 60–110 region in the ^{13}C-nmr spectrum. To confirm the position of the carboxyl group, selective INEPT nmr experiments, similar to those for **1**, were carried out. Irradiation ($^3J_{CH}$=4 Hz) of the methyl proton at δ 1.23 (H-30), which correlated with δ 19.0 (q, C-30) (10) in the ^1H-^{13}C HETCOR spectrum, selectively enhanced carbons at δ 42.5 (s, C-20) and 185.3 (s, C-29). Thus, compound **2** was identified as the new natural product, 3-oxo-olean-12-en-29-oic acid. However, this derivative has been obtained previously by oxidation of katonic acid [**3**] (13).

Compound **3** resembled compounds **1** and **2** in its spectral data (ir, ^1H nmr, ^{13}C nmr and eims), indicating that it was also an olean-12-enoic acid-type triterpene. ^{13}C-nmr chemical shifts at δ 49.2 (d, C-5) and 75.1 (d) revealed that a hydroxyl group was attached at C-3 with α configuration (10). The position of a carboxyl group was confirmed at C-29 by the selective INEPT experiment using the same parameters as those carried out for **1** and **2**. Irradiation of H-30 (δ 1.46) resulted in enhancements of C-20 (s, δ 42.8) and C-29 (s, δ 181.1). Therefore, compound **3** was identified as 3α-hydroxyolean-12-en-29-oic acid (katonic acid), which has already been isolated from the heartwood of *S. koetjape* (6, 13).

Three known sesquiterpenes were identified as (−)-alloaromadendrene, (−)-caryophyllene oxide, and (+)-spathulenol, respectively, by comparison with published physical and spectroscopic data ([α]D, ir, ^1H nmr, ^{13}C nmr, and eims) (14–17). Although these types of sesquiterpenes were reported from some genera (18, 19) in Meliaceae, they have been also found more commonly as constituents of numerous *Eucalyptus* oils (20).

The cytotoxic activity of all six of the isolates obtained in this investigation was evaluated with ten human cancer cell lines and murine lymphocytic leukemia in cell

culture (P-388) (21–23). Also, potential antimitotic activity was evaluated in an astrocytoma (ASK) assay (24). As summarized in Table 1, compounds **2** and **3** (oleanane-type triterpenes) were cytotoxic against several of the cell lines in which they were evaluated; the most intense response was observed with P-388 cells. In contrast, compound **1** (an oleanane-type ring-A secotriterpene) and the three known sesquiterpenes were inactive against all of the cancer cell lines utilized (ED_{50} >20 μg/ml). None of the test compounds showed demonstrable antimitotic activity as judged by the ASK assay. Among recently reported cytotoxic triterpenes, activity has been demonstrated for the following cell lines: KB and BL-6 (25) and P-388 (26) (oleanane-type); KB (27), P-388 (26), and KB, P-388, L-1210, A-549, HCT-8, and MCF-7 (28) (ursane-type); Co-115 (29) (lupane-type); and A-549 (30) (friedelane-type). Interestingly, all cytotoxic triterpenes of the above examples possessed either a keto group or a carboxyl group, as was the case for compounds **2** and **3**. The present investigation has demonstrated that fission of ring A negated cytotoxicity against the cancer cell lines employed, suggesting that this area of the molecule is critical for activity. In contrast, it has been noted that oxidative fission of the triterpene A ring often leads to derivatives that exhibit antibacterial activity (11).

TABLE 1. Cytotoxic Activity of Triterpenes **2** and **3**.[a]

Compound	Cell line										
	A-431	BC1	Col2	HT-1080	KB	KB-V1	LNCaP	Lu1	Mel2	ZR-75-1	P-388
2	>20	>20	>20	>20	4.9	8.5	10	10	12	5.2	0.61
3	12	7.8	>20	19	8.3	15	10	11	12	2.2	0.11

[a]Results are expressed as ED_{50} values (μg/ml). Abbreviations: A-431, epidermoid carcinoma; BC1, breast; Col2, colon; HT-1080, sarcoma; KB, nasopharyngeal carcinoma; KB-V1, drug-resistant KB; LNCaP, prostate; Lu1, lung; Mel2, melanoma; ZR-75-1, breast; P-388, murine lymphocytic leukemia.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points (uncorrected) were determined on a Kofler hot-stage apparatus. Optical rotations were measured with a Perkin-Elmer Model 241 polarimeter. Ir spectra were obtained with a Nicolet MX-1 interferometer. ^{1}H-nmr and ^{13}C-nmr spectra were measured with TMS as internal standard, employing a Varian XL-300 instrument operating at 300 MHz and 75.6 MHz, respectively. ^{1}H-^{1}H COSY and ^{1}H-^{13}C HETCOR nmr experiments were also performed on a Varian XL-300, using standard Varian pulse sequences. Selective INEPT nmr experiments were conducted on a Nicolet NT-360 spectrometer performing at 90.8 MHz. Eims spectra were taken on a Varian MAT 112S double focusing mass spectrometer at 70 eV. High resolution eims were obtained using a Finnegan MAT 90 instrument.

CYTOTOXIC ASSAYS.—The Et_2O extract of *S. koetjape* stems was tested for cytotoxic activity against eight cell lines: BC1 (human breast cancer), Col2 (human colon cancer), HT-1080 (human fibrosarcoma), KB (human nasopharyngeal carcinoma), KB-V1 (vinblastine-resistant KB), Lu1 (human lung cancer), Mel2 (human melanoma), and P-388 (murine lymphocytic leukemia), by procedures as described previously (21–23). The cytotoxic activity of chromatographic fractions obtained from the Et_2O extract was monitored by P-388 cytotoxicity, for which the extracts showed the greatest potency. Pure compounds isolated in this manner were tested for cytotoxicity against the above-mentioned cell lines as well as three additional cell lines, A-431 (human epidermoid carcinoma), LNCaP (human prostate cancer), and ZR-75-1 (human breast cancer). These evaluations were performed by published procedures (22,23). In addition, antimitotic potential was assessed using cultured ASK cells (24). Each test compound was evaluated in duplicate at five concentrations (20.0, 4.0, 0.8, 0.16, and 0.32 μg/ml).

PLANT MATERIAL.—The stems of *S. koetjape* were collected in August 1989, in Saraburi Province,

Thailand. A voucher specimen representing this collection has been deposited at the Herbarium of the Royal Forestry Department, Bangkok, Thailand.

EXTRACTION AND ISOLATION.—The dried stems of *S. koetjape* (21 kg) were extracted with MeOH (60 liters) at room temperature to give a residue (2.0 kg) on removal of solvent under vacuum. This extract was taken up in MeOH (500 ml), and the resultant suspension in H_2O (4 liters) was treated with Et_2O (3 liters×4). The Et_2O layer was concentrated to dryness, and the residue (230 g) was subjected to chromatography over a Si gel column with $CHCl_3$ and $CHCl_3$/MeOH as eluents. Additional Si gel cc was carried out on the first fraction (8.3 g) obtained from the initial column, to afford (−)-alloaromadendrene (1.1 g, 0.0052% w/w) (extracted with *n*-heptane), (−)-caryophyllene oxide (0.77 g, 0.0037% w/w) and (+)-spathulenol (1.3 g, 0.0062% w/w) (extracted with toluene), and 3-oxo-olean-12-en-29-oic acid [**2**] (0.96 g, 0.0046% w/w) [extracted with $CHCl_3$-Me_2CO (5:1)]. From the second fraction (33.5 g) of the original cc, katonic acid [**3**] (19.5 g, 0.093% w/w) was eluted by further cc over Si gel with $CHCl_3$-MeOH (20:1). Likewise from the third fraction (37.9 g) of the original cc, **1** (23.2 g, 0.11% w/w) was obtained by subsequent cc using $CHCl_3$-MeOH (5:1) as solvent. Compound **1** was finally purified by crystallization from MeOH, and compounds **2** and **3** by crystallization from Me_2CO.

TABLE 2. ^{13}C-nmr Spectra of Triterpenes **1–3**.[a]

Carbon	Compound			
	1[b]	**1** methyl ester[c]	**2**[c]	**3**[b]
C-1	24.9 t	24.4 t	39.2 t	33.7 t
C-2	31.6 t	31.3 t	34.1 t	26.4 t
C-3	176.3 s	174.4 s	217.8 s	75.1 d
C-4	147.9 s	147.3 s	47.3 s	37.9 s
C-5	38.1 d	37.8 d	55.1 d	49.2 d
C-6	29.3 t	28.4 t	19.6 t	18.6 t
C-7	35.0 t	34.0 t	32.0 t	33.0 t
C-8	39.8 s	39.5 s	39.7 s	40.3 s
C-9	50.3 d	50.4 d	46.7 d	47.7 d
C-10	39.4 s	39.1 s	36.6 s	37.4 s
C-11	24.0 t	23.6 t	23.6 t	23.9 t
C-12	122.7 d	122.4 d	122.5 d	123.1 d
C-13	145.0 s	144.2 s	143.9 s	144.6 s
C-14	42.4 s	42.0 s	41.7 s	42.0 s
C-15	26.5 t	26.0 t	25.9 t	26.4 t
C-16	27.3 t	26.9 t	26.8 t	27.3 t
C-17	32.4 s	31.9 s	32.3 s	32.8 s
C-18	48.7 d	48.2 d	45.9 d	46.5 d
C-19	43.5 t	42.8 t	40.1 t	41.5 t
C-20	44.3 s	44.2 s	42.5 s	42.8 s
C-21	31.8 t	31.2 t	28.7 t	29.9 t
C-22	39.0 t	38.3 t	35.7 t	36.5 t
C-23	113.8 t	113.5 t	26.4 q	29.3 q
C-24	23.9 q	23.4 q	21.4 q	22.8 q
C-25	19.8 q	19.4 q	15.1 q	15.7 q
C-26	17.0 q	16.8 q	16.6 q	17.1 q
C-27	26.1 q	25.7 q	25.7 q	26.0 q
C-28	28.6 q	28.4 q	28.1 q	28.5 q
C-29	29.1 q	28.1 q	185.3 q	181.1 q
C-30	179.4 s	177.5 s	19.0 q	20.0 q
3-COOMe	—	51.4 q	—	—
30-COOMe	—	51.4 q	—	—

[a]Measured at 75.6 MHz, δ TMS=0.
[b]Obtained in pyridine-d_5.
[c]Obtained in $CDCl_3$.

Koetjapic acid [**1**].—Mp 296–298° (colorless prisms, MeOH); $[\alpha]^{23}D$ +114.2° (*c*=0.12, MeOH); ir ν max (KBr) 3440, 2980–2880, 1700, 1460, 1390, 1280, 1230 cm^{-1}; ^1H nmr (C_5D_5N) δ 0.89 (3H, s, H-28), 0.95 (3H, s, H-26), 1.04 (3H, s, H-25), 1.27 (3H, s, H-27), 1.39 (3H, s, H-29), 1.83 (3H, s, H-24), 4.89 (1H, s, H-23), 4.98 (1H, s, H-23), 5.44 (1H, br s, H-12); ^{13}C nmr see Table 2; eims *m/z* (rel. int.) $[M]^+$ 470 (5), 248 (100), 147 (17), 107 (30), 81 (34), 55 (45), 41 (41); hreims *m/z* $[M]^+$ 470.3398 (calcd for $C_{30}H_{46}O_4$, 470.3396).

Koetjapic acid dimethyl ester.—Compound **1** (100 mg) was treated with CH_2N_2 to give a dimethyl ester (105 mg): viscous solid; $[\alpha]^{25}D$ +112.7° (*c*=0.52, $CHCl_3$); ir ν max (KBr) 3430, 2980–2870, 1730, 1450, 1170 cm^{-1}; ^1H nmr ($CHCl_3$) δ 0.79 (3H, s, H-28), 0.94 (3H, s, H-25), 1.02 (3H, s, H-26), 1.13 (3H, s, H-27), 1.16 (3H, s, H-29), 1.75 (3H, s, H-24), 3.65 (3H, s, ester Me), 3.69 (3H, s, ester Me), 4.68 (1H, s, H-23), 4.87 (1H, s, H-23), 5.29 (1H, br s, H-12); ^{13}C nmr see Table 2; eims *m/z* (rel. int.) $[M]^+$ 498 (47), 416 (17), 262 (100).

3-Oxo-olean-12-en-29-oic acid [**2**].—Mp 255–256° (colorless needles, Me_2CO); $[\alpha]^{23}D$ +85.3° (*c*=0.59, $CHCl_3$) [lit (13) mp 256–260°, $[\alpha]D$ +80° (*c*=1.3)]; ir ν max (KBr) 3430, 2980–2860, 1730, 1720, 1710, 1460, 1380, 1210, 1110 cm^{-1}; ^1H nmr ($CDCl_3$) δ 0.87 (3H, s, H-28), 1.02 (3H, s, H-26), 1.06 (3H, s, H-24), 1.07 (3H, s, H-25), 1.10 (3H, s, H-23), 1.15 (3H, s, H-27), 1.23 (3H, s, H-30), 5.25 (1H, br s, H-12); ^{13}C nmr see Table 2; eims *m/z* (rel. int.) $[M]^+$ 454 (3), 410 (4), 248 (100), 187 (60), 173 (52), 81 (34), 55 (44), 43 (33), 41 (49); hreims *m/z* $[M]^+$ 454.3444 (calcd for $C_{30}H_{46}O_3$, 454.3447).

Katonic acid [**3**].—Mp 258–260° (colorless needles, Me_2CO); $[\alpha]^{23}D$ +47.9° (*c*=0.68, $CHCl_3$) [lit. (6) mp 285–287°, $[\alpha]D$ +47°]; ir ν max (KBr) 3450, 2980–2880, 1700, 1470, 1460, 1380, 1210, 1070 cm^{-1}; ^1H nmr (C_5D_5N) δ 0.93 (3H, s, H-24), 0.96 (3H, s, H-28), 1.00 (3H, s, H-25), 1.04 (3H, s, H-26), 1.13 (3H, s, H-27), 1.21 (3H, s, H-23), 1.46 (3H, s, H-30), 2.50 (1H, t, *J*=13.9 Hz, H_{ax}-19), 3.61 (1H, br s, H-3), 5.32 (1H, br s, H-12); ^{13}C nmr see Table 2; eims *m/z* (rel. int.) $[M]^+$ 456 (3), 438 (3), 418 (7), 248 (94), 173 (48), 136 (81), 81 (40), 55 (75), 43 (100), 41 (100).

(−)-Alloaromadendrene.—Colorless oil; $[\alpha]^{23}D$ −26.5° (*c*=4.0, $CHCl_3$) [lit. (14) $[\alpha]D$ −12° (*c*=1.65, $CHCl_3$)]; ir, ^1H nmr, ^{13}C nmr, and eims see De Rosa *et al.* (14).

(−)-Caryophyllene oxide.—Mp 59–61° (colorless prisms); $[\alpha]^{23}D$ −54.0° (*c*=1.36, $CHCl_3$) [lit. (15) mp 61–63°, $[\alpha]^{20}D$ −71.6° ($CHCl_3$)]; ir and eims see Maurer and Grieder (15); ^1H nmr and ^{13}C nmr see Krebs *et al.* (16).

(+)-Spathulenol.—Colorless oil; $[\alpha]^{25}D$ +7.4° (*c*=1.2, $CHCl_3$) [lit. (17) $[\alpha]D$ +6.5° (*c*=1.97, $CHCl_3$)]; ^1H nmr and ^{13}C nmr see Krebs *et al.* (16); ir and eims see Surburg and Mondon (17).

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LITERATURE CITED

1. N. Kaneda, H. Chai, J.M. Pezzuto, A.D. Kinghorn, N.R. Farnsworth, P. Tuchinda, J. Udchachon, T. Santisuk, and V. Reutrakul, *Planta Med.*, (1992), in press.
2. J.C. Uphof, "Dictionary of Economic Plants," 2nd ed., Verlag von J. Cramer, Lehre, Germany, 1968.
3. I.H. Burkill, "Dictionary of the Economic Products of the Malay Peninsula," Ministry of Agriculture and Cooperatives, Kuala Lumpur, Malaysia, 1966, Vol. II.
4. S. Wasuwat, "A List of Thai Medicinal Plants. Report No. 1 on Research Project 17," Appl. Sci. Res. Corp. Thailand, Bangkok, Thailand, 1967.
5. R. Kleiman and K.L. Payne-Wahl, *J. Am. Oil Chem. Soc.*, **61**, 1836 (1984).
6. F.E. King and J.W.W. Morgan, *J. Chem. Soc.*, 4738 (1960).
7. K.Y. Sim and H.T. Lee, *Phytochemistry*, **11**, 3341 (1972).
8. R.G. Powell, K.L. Mikolajczak, B.W. Zilkowski, E.K. Mantus, D. Cherry, and J. Clardy, *J. Nat. Prod.*, **54**, 241 (1991).
9. A. Bax, *J. Magn. Reson.*, **57**, 314 (1984).
10. T.K. Chen, D.C. Ales, N.C. Baenziger, and D.F. Wiemer, *J. Org. Chem.*, **48**, 3525 (1983).
11. W.J. Baas, *Phytochemistry*, **24**, 1875 (1985).
12. J. de Pascual Teresa, J.G. Urones, I.S. Marcos, P. Basabe, M.J. Sexmero Cuadrado, and R. Fernandez Moro, *Phytochemistry*, **26**, 1767 (1987).

13. K.L. Gaul, K.G. Lewis, B.W. Skelton, and A.H. White, *Aust. J. Chem.*, **38**, 1371 (1985).
14. S. De Rosa, S. De Stefano, P. Scarpelli, and N. Zavodnik, *Phytochemistry*, **27**, 1875 (1988).
15. B. Maurer and A. Grieder, *Helv. Chim. Acta*, **60**, 2177 (1977).
16. H.C. Krebs, J.V. Rakotoarimanga, and G.G. Habermehl, *Magn. Reson. Chem.*, **28**, 124 (1990).
17. H. Surburg and A. Mondon, *Chem. Ber.*, **114**, 118 (1981).
18. A. Nanda, M.A. Iyengar, C.S. Narayan, and D.R. Kulkarni, *Fitoterapia*, **58**, 189 (1987).
19. C.P. Wang and H. Kameoka, *Nippon Nogei Kagaku Kaishi*, **52**, 297 (1978).
20. H. Nishimura and M. Calvin, *J. Agric. Food Chem.*, **27**, 432 (1979).
21. M. Arisawa, J.M. Pezzuto, C. Bevelle, and G.A. Cordell, *J. Nat. Prod.*, **47**, 453 (1984).
22. H. Jayasuriya, J.D. McChesney, S.M. Swanson, and J.M. Pezzuto, *J. Nat. Prod.*, **52**, 325 (1989).
23. G. Blaskó, H.L. Shieh, J.M. Pezzuto, and G.A. Cordell, *J. Nat. Prod.*, **52**, 1363 (1989).
24. S.M. Swanson, J-X. Jiang, N.J. de Souza, and J.M. Pezzuto, *J. Nat. Prod.*, **51**, 929 (1988).
25. M. Arisawa, N. Morita, T. Ota, M. Maeda, S. Odashima, S. Shimizu, and S. Koshimura, in: "Abstracts." International Research Congress on Natural Products, Chicago, Illinois, July 21–26, 1991, Abstract P-122.
26. A. Numata, P. Yang, C. Takahashi, R. Fujiki, M. Nabae, and E. Fujita, *Chem. Pharm. Bull.*, **37**, 648 (1989).
27. H. Hori, G.-M. Pang, K. Harimaya, Y. Iitaka, and S. Inayama, *Chem. Pharm. Bull.*, **35**, 2125 (1987).
28. K-H. Lee, Y-M. Lin, T-S. Wu, D-C. Zhang, T. Yamagishi, T. Hayashi, I.H. Hall, J-J. Chang, R-Y. Wu, and T-H. Yang, *Planta Med.*, **54**, 308 (1988).
29. F.A. Tomás-Barberán and K. Hostettmann, *Planta Med.*, **54**, 266 (1988).
30. H. Nozaki, Y. Matsuura, S. Hirono, R. Kasai, J-J. Chang, and K-H. Lee, *J. Nat. Prod.*, **53**, 1039 (1990).

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