Plant Anticancer Agents, XLVIII. New Cytotoxic Flavonoids from Muntingia calabura Roots

Norito Kaneda, John M. Pezzuto, D. Doel Soejarto, A. Douglas Kinghorn, Norman R. Farnsworth, Thawatchai Santisuk, Patoomratana Tuchinda, Jinda Udchachon, and Vichai Reutrakul

J. Nat. Prod., **1991**, 54 (1), 196-206• DOI:
10.1021/np50073a019 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 54, No. 1, pp. 196-206, Jan-Feb 1991

PLANT ANTICANCER AGENTS, XLVIII. NEW CYTOTOXIC FLAVONOIDS FROM *MUNTINGIA CALABURA* ROOTS[1,2]

NORITO KANEDA, JOHN M. PEZZUTO, D. DOEL SOEJARTO, A. DOUGLAS KINGHORN,* NORMAN R. FARNSWORTH,

Program for Collaborative Research in the Pharmaceutical Sciences, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois 60612

THAWATCHAI SANTISUK,

The Forest Herbarium, Royal Forestry Department, Bangkok 10900, Thailand

PATOOMRATANA TUCHINDA, JINDA UDCHACHON, and VICHAI REUTRAKUL

Department of Chemistry, Faculty of Science, Mahidol University, Bangkok 10400, Thailand

ABSTRACT.—From a cytotoxic Et_2O-soluble extract of *Muntingia calabura* roots, twelve new flavonoids were isolated, constituting seven flavans **1–7**, three flavones **8**, **10**, and **12**, and two biflavans **9** and **11**. The structures of compounds **1–12** were established by the interpretation of spectral data, with the nmr assignments of these constituents being based on ^1H-^1H COSY, ^1H-^{13}C HETCOR, and selective INEPT experiments. This is the first report of the occurrence of 7,8-di-*O*-substituted flavans, biflavans, and flavones. Most of the isolates demonstrated cytotoxic activity when tested against cultured P-388 cells, with the flavans being more active than the flavones. Furthermore, certain of these structurally related flavonoids exhibited somewhat selective activities when evaluated with a number of human cancer cell lines.

Roots of *Muntingia calabura* L. (Elaeocarpaceae) were investigated as part of a continuing project to discover novel antineoplastic agents of plant origin (1). Samples were collected in Thailand for preliminary screening and in the Philippines for detailed phytochemical investigation. This tree species, introduced from tropical America to southeast Asia, does not appear to have been used for the treatment of cancer historically, but its various plant parts have been utilized for other medicinal purposes. In Indochina (Cambodia), the roots are mixed with other drugs and employed as an emmenagogue and for the treatment of liver diseases (2–4). Infusions of the flowers are drunk in east Asia in cases of headaches and incipient colds (4–6) and are consumed in both east Asia and South America for antihysteric, antidyspeptic, diaphoretic (4,7), and antispasmodic purposes (4, 7–10). This same type of infusion is also drunk as a tranquilizer or a tonic in Colombia (9).

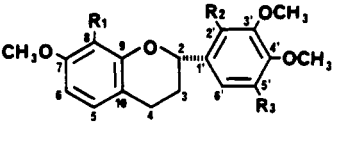
Despite the widespread use of this plant, previous phytochemical investigations have been few and constitute investigations on its seed oil (11) and tannin (12) constituents. In the present study, twelve new flavonoids were isolated from *M. calabura* roots, and reported herein are details of the isolation, structure elucidation, and cytotoxic activity of these compounds.

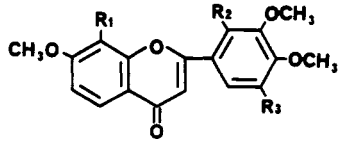
RESULTS AND DISCUSSION

For compound **1**, a molecular formula of $C_{18}H_{20}O_5$ was consistent with its ms and ^{13}C-nmr spectral data. The presence of a simple benzenoid chromophore having an unconjugated aromatic system was suggested by observed absorption maxima at 227 and 280 nm in the uv spectrum and at 1600 and 1510 cm^{-1} (benzene ring) in the ir spectrum. Compound **1** exhibited 18 carbons including three methoxy carbons at δ 55.1

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

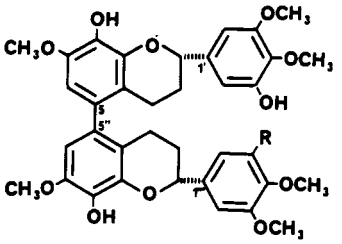
[2]Dedicated to Professor G.B. Marini Bettolo on the occasion of his 75th birthday.





1 $R_1=R_2=H$, $R_3=OH$
2 $R_1=R_3=OMe$, $R_2=H$
3 $R_1=R_3=OMe$, $R_2=OH$
4 $R_1=OMe$, $R_2=H$, $R_3=OH$
5 $R_1=OH$, $R_2=H$, $R_3=OMe$
6 $R_1=R_2=OH$, $R_2=OMe$
7 $R_1=R_3=OH$, $R_2=H$

8 $R_1=R_3=OMe$, $R_2=H$
10 $R_1=OMe$, $R_2=H$, $R_3=OH$
12 $R_1=R_3=OH$, $R_2=H$



9 R=OMe
11 R=OH

(q), 55.7 (q), and 60.7 (q) in its ^{13}C-nmr spectrum, which indicated that its skeleton might be that of a flavonoid. Resonances occurring at δ 24.3 (t), 29.9 (t), and 77.7 (d), constituting a C-3 unit, indicated that **1** was a flavan (13). In the ^{1}H-nmr spectrum, one typical aromatic ABX pattern (3H) was observed at δ 6.46 (dd, $J=9.0$, 2.5 Hz), 6.48 (d, $J=2.5$ Hz), and 6.96 (d, $J=9.1$ Hz), and one meta-coupled ($J=1.8$ Hz) nonequivalent proton system also appeared as doublets at δ 6.56 and 6.66. By interpretation of the ^{13}C chemical shifts of three methoxyl groups (55.1, 55.7, and 60.7) (14) and of the eims spectral fragmentation (*m/z* 137, 167, 180) (15), ring B was confirmed as 5′-hydroxy-3′,4′-dimethoxyphenyl. The position of one methoxyl group in ring A was determined as occurring at C-7 by analysis of its ^{13}C-nmr chemical shifts and comparison with model compounds (16,17) and by using the selective INEPT nmr experiment, which identifies vicinal ^{13}C-^{1}H coupling (18). In the latter, irradiation ($^3J_{CH}=8$ Hz) of the meta-coupled proton at δ 6.48 (d, $J=2.5$ Hz) resulted in enhancements of carbons at δ 113.8 (C-10) and 107.3 (C-6). The conformation of **1** was suggested by coupling constants of H-2 (dd, $J=10.2$, 2.4 Hz) in the ^{1}H-nmr spectrum, which indicated that the B ring was equatorially substituted at the C-2 position (19–21). The absolute configuration of compound **1** was assigned as 2*S* by the negative cd absorption maximum at 284 nm ($\Delta\epsilon$ −1.3), by analogy with previous cd spectral observations (22–25).

Compound **2** exhibited uv (λ max 229, 274 nm) and ir (ν max 1590, 1500 cm^{-1}) spectral data similar to those of **1**, suggesting that it was also a flavan. Its hreims spectrum (*m/z* 360.1563), as well as its ^{1}H- and ^{13}C-nmr spectra, indicated that the molecular formula was $C_{20}H_{24}O_6$. In the ^{1}H-nmr spectrum, two ortho-coupled protons and two singlet aromatic protons (2H) were apparent at δ 6.50 (d, $J=8.5$ Hz), 6.77 (d, $J=8.7$ Hz), and 6.68 (s), respectively. It was observed by analysis of the ^{1}H- and ^{13}C-nmr spectra that five methoxyl groups were substituted in rings A and B. The placements of these methoxyl groups were confirmed as 7,8-dimethoxy in ring A and 3′,4′,5′-trimethoxy in ring B by the ^{13}C chemical shift of two methoxy carbons (δ 60.6

and 60.7) (14) and eims spectral fragmentation peaks (*m/z* 179, 181, 194) (15). From the coupling constant (dd, $J = 9.8$, 2.3 Hz) at δ 5.05 in the ^{1}H-nmr spectrum, the H-2 proton was confirmed as axial (19). The absolute configuration of compound **2** was also determined as 2*S* by measurement of its cd spectrum ($\Delta\epsilon$ −1.0 at λ max 283 nm) (22).

Compound **3** ($C_{20}H_{24}O_7$) exhibited spectral data (uv, ir, cd, ^{1}H and ^{13}C nmr) similar to those of compounds **1** and **2**, except that it possessed a somewhat different substitution pattern. From the eims fragmentation pathway (*m/z* 167, 197, 210) (15) and ^{13}C chemical shifts of the three methoxy carbons at δ 60.8, 60.9, and 61.2 (14), it was evident that compound **3** was 7,8-dimethoxy-substituted in ring A and either 2′-hydroxy-3′,4′,5′-trimethoxy- or 5′-hydroxy-2′,3′,4′-trimethoxy-substituted in ring B. To confirm the substitution pattern in ring B, a selective INEPT nmr experiment was carried out (18). Irradiation ($^3J_{CH} = 5$ Hz) of the hydroxy proton at δ 5.75 resulted in enhancements of carbons at δ 121.8 (C-1′), 140.0 (C-2′), and 140.1 (C-3′). Therefore, compound **3** was established as (2*S*)-2′-hydroxy-7,8,3′,4′,5′-pentamethoxyflavan.

Compounds **4–7** resembled compounds **1–3** in their spectral data (uv, ir, cd, ^{1}H and ^{13}C nmr, eims) but showed lesser degrees of methoxylation than compounds **2** and **3**. For compound **4** ($C_{19}H_{22}O_6$), four methoxy protons, one ortho-coupled aromatic proton, and one meta-coupled aromatic proton, in turn, were observed in the ^{1}H-nmr spectrum at δ 3.83–3.88 (12H), 6.48 (d, $J = 8.5$ Hz), and 6.74 (d, $J = 8.5$ Hz) and 6.59 (d, $J = 1.8$ Hz) and 6.65 (d, $J = 1.9$ Hz). The positions of the four methoxyl groups in rings A and B were resolved as being at C-7, C-8, C-3′, and C-4′ by further interpretation of ^{13}C nmr (δ 55.4, 55.8, 60.3, 60.4) and eims (*m/z* 166, 167, 180) spectra. Thus, compound **4** was determined as (2*S*)-5′-hydroxy-7,8,3′,4′-tetramethoxyflavan.

For compound **5** ($C_{19}H_{22}O_6$), the main fragment peaks (*m/z* 179, 181, 194) in the eims spectrum were identical to those of **2**. The ^{1}H-nmr spectra of these two isolates were also similar, except that one hydroxy proton at δ 5.64 (s) appeared for compound **5**, in place of the methoxy protons at δ 3.89 (s) for compound **2**. The position of this hydroxyl group was placed at C-8, because the C-7 methoxy carbon of **2** persisted in the ^{13}C-nmr spectrum of **5** (56.0), while the C-8 methoxy resonance of **2** was no longer present. Therefore, compound **5** was elucidated as (2*S*)-8-hydroxy-7,3′,4′,5′-tetramethoxyflavan.

Compound **6** ($C_{19}H_{22}O_7$) was considered as an analogue of **3** from its eims fragmentation pathway (*m/z* 197, 210). In the ^{1}H-nmr spectrum, two hydroxy protons were observed at δ 5.70 (s) and 6.00 (s). In a selective INEPT nmr experiment similar to that carried out for **3**, the positions of these hydroxyl groups were confirmed at C-8 and C-2′, respectively. Irradiation ($^3J_{CH} = 5$ Hz) of the hydroxy protons at δ 5.70 resulted in enhancements of carbons at δ 133.8 (C-8), 142.6 (C-9), and 145.3 (C-7), while an analogous irradiation at δ 6.00 gave the corresponding enhancements of carbons at δ 121.3 (C-1′), 139.9 (C-2′), and 140.0 (C-3′). Thus, compound **6** was established as (2*S*)-8,2′-dihydroxy-7,3′,4′,5′-tetramethoxyflavan.

For compound **7** ($C_{18}H_{20}O_6$), its eims spectral data (*m/z* 167, 180) suggested that the substitution pattern in ring B was the same as in **1** and **4**. On the observation of three methoxy carbons (δ 55.9, 56.4, 60.9) in the ^{13}C-nmr spectrum, and one ortho-coupled ($J = 8.5$ Hz) proton system and two hydroxy protons at δ 5.49 (s) and 5.88 (s) in the ^{1}H-nmr spectrum, the positions of these two hydroxyl groups were confirmed at C-8 and C-5′. Therefore, compound **7** was determined as (2*S*)-8,5′-dihydroxy-7,3′,4′-trimethoxyflavan.

For compound **8** ($C_{20}H_{20}O_7$), the presence of an aromatic ring conjugated to a carbonyl group was suggested by absorption maxima at 241 and 316 nm in its uv spectrum and at 1640 (C=O), 1600, and 1500 cm^{-1} (benzene ring) in its ir spectrum. In the ^{1}H-

nmr spectrum, five methoxy proton, two singlet aromatic proton, and one ortho-coupled proton systems were observed, in turn, at δ 3.94–4.05 (15H), 6.72 (1H, s), 7.20 (2H, s), 7.06 (d, $J = 8.8$ Hz), and 7.94 (d, $J = 8.8$ Hz), suggesting that **8** was a flavone, co-occurring with the flavan **2**, of identical substitution pattern. This was confirmed by the ^{13}C-nmr spectrum showing two methoxy signals at δ 60.9 and 61.3. Therefore, compound **8** was determined as 7,8,3′,4′,5′-pentamethoxyflavone.

The spectral data (uv, ir, ^{1}H and ^{13}C nmr) of compounds **10** and **12** were almost identical to those of **8**, except for variations in the number of methoxyl groups (**10**, four methoxyls; **12**, three methoxyls). In the same way as for **1–8**, compounds **10** and **12** were elucidated as 5′-hydroxy-7,8,3′,4′-tetramethoxyflavone ($C_{19}H_{18}O_7$) and 8,5′-dihydroxy-7,3′,4′-trimethoxyflavone ($C_{18}H_{16}O_7$), respectively. As a consequence of selective INEPT nmr experiments carried out on compounds **1–8**, **10**, **12**, it was observed that irradiation of a given methoxy proton clearly enhanced only its attached carbon, using an experimental parameter of $^{3}J_{CH} = 4$ Hz.

Compounds **9** and **11** indicated uv and ir spectral patterns similar to those of flavans **1–7**. The respective eims fragmentation patterns (**9**, *m/z* 167, 181, and 676; **11**, *m/z* 167 and 662) revealed that they were biflavans and also suggested that **9** was comprised of two units represented by flavans **5** and **7**, while **11** was a dimer of flavan **7**. In their ^{1}H- and ^{13}C-nmr spectra, companion peaks of similar resonance were observed for compounds **9** and **11**. The largest difference in ^{13}C chemical shift of these companion peaks was 0.7 ppm in **9** and 0.6 ppm in **11**. Consequently, it was considered that these companion peaks were indicative of the existence of stereoisomerism. After a ^{1}H-^{13}C HETCOR nmr experiment, both **9** and **11** were found to contain a biphenyl unit, linked at C-5 and C-5″. It is known that biphenyls and analogous biaryl derivatives cause stereoisomerism resulting from axes of chirality and from helicity (26,27). In the case of compounds **9** and **11**, the biphenyl moiety is symmetric, for which a configurationally stable chiral axis does not exist. Thus, the stereochemistry of biphenyls may be described as *M* and *P* in terms of helicity (26). The absolute configurations of **9** and **11** were determined as 2*S* and 2″*S* by strong negative absorption maxima at 289 nm ($\Delta\epsilon -2.3$) and at 288 nm ($\Delta\epsilon -1.3$), respectively, in their cd spectra. Therefore, compound **9** ($C_{37}H_{40}O_{12}$) was determined as a mixture of the two diastereomers [(*M*),(2*S*),(2″*S*)- and (*P*),(2*S*),(2″*S*)-] of 8,8″-5′-trihydroxy-7,7″-3′,3‴-4′,4‴-5‴-heptamethoxy-5,5″-biflavan, and compound **11** ($C_{36}H_{38}O_{12}$) was also deduced to be a mixture of the two diastereomers [(*M*),(2*S*),(2″*S*)- and (*P*),(2*S*),(2″*S*)-] of 8,8″-5′,5‴-tetrahydroxy-7,7″-3′,3‴-4′,4‴-hexamethoxy-5,5″-biflavan.

It is generally considered that flavans arise from double reductions of analogous flavanones, because they co-occur with flavanones of identical substitution pattern, in spite of often possessing most unusual patterns of substitution (23). In this study, flavones were indeed found to co-occur with flavans of the same substitution pattern.

As summarized in Table 1, all of the isolates except compound **8** were found to demonstrate cytotoxic activity with cultured P-388 cells; the flavans **1–7** were more active than the structurally related flavones **10** and **12** in this test system. Flavans **1–7** appeared somewhat selectively active toward melanoma (Me12) and KB cells. Moreover, relative to the parent KB cell line, enhanced activity could be demonstrated with the vincristine-resistant KB (KB-V) cell line, especially in the case of compounds **2** and **5**. In contrast, compound **3** (hydroxylated at the 2′-position) and compounds **9** and **11** (biflavans) were generally cytotoxic and not preferentially active against KB-V cells. The structurally related flavones **10** and **12** were not active against KB (and KB-V) or melanoma cells. However, marginal activity was observed with the human colon (Co12) cell line, which again suggests some selectivity. A deeper understanding of the

TABLE 1. Cytotoxicity Data of Compounds **1–12**.[a]

Compound	Structual type	Cell lines[b]							
		BC1	HT	Lu1	Me12	Co12	KB	KB-V	P-388
1	flavan	>20	>20	>20	14.6	>20	9.4	13.3	5.9
2	flavan	>20	>20	>20	8.9	15.8	13.3	2.1	5.4
3	flavan	10.9	3.3	13.5	9.7	12.0	3.4	6.2	4.9
4	flavan	>20	>20	>20	9.0	>20	15.5	12.3	2.0
5	flavan	>20	>20	>20	9.2	>20	11.8	3.9	3.0
6	flavan	>20	>20	>20	14.5	>20	10.2	>20	2.3
7	flavan	>20	>20	>20	10.6	>20	13.8	11.1	4.1
8	flavone	NT[c]	NT[c]	NT[c]	NT[c]	NT[c]	NT[c]	NT[c]	NT[c]
9	biflavan	12.0	5.5	12.4	10.2	6.2	2.2	8.3	3.7
10	flavone	>20	>20	>20	>20	15.2	>20	>20	11.9
11	biflavan	16.0	5.0	15.6	8.7	9.0	5.2	12.6	4.8
12	flavone	>20	>20	>20	>20	5.9	>20	>20	16.7

[a]Results are expressed as ED_{50} values (μg/ml).

[b]BC1 human breast cancer; HT (HT-1080), human fibrosarcoma; Lu1, human lung cancer; Me12, human melanoma; Co12, human colon cancer; KB, human nasopharyngeal carcinoma; KB-V, vincristine-resistant KB; P-388, murine lymphocytic leukemia.

[c]NT: not tested, since compound was difficult to dissolve in dimethyl sulfoxide.

mechanisms of these differential activities could contribute toward site-specific chemotherapeutic strategies.

Previous reports (28–31) have pointed to the cytotoxic activity of methoxylated flavonoids (flavones, isoflavones, flavanones, and flavonols). The cytotoxicity of flavans **1–7** illustrates that the activity of flavonoids is due to methoxyl and/or hydroxyl groups in the structures and also shows that a C-3 carbonyl group is not necessary to elicit this type of activity.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points (uncorrected) were determined on a Kofler hot-stage apparatus. Optical rotations were taken with a Perkin-Elmer Model 241 polarimeter. Uv spectra were obtained on a Beckman DU-7 spectrometer, and cd spectra were measured on a JASCO J-40A automatic recording spectropolarimeter. 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. Mass spectra were taken on a Varian MAT 112S double focusing mass spectrometer at 80 eV. High resolution ei mass spectra were obtained using a Finnegan MAT 90 instrument.

CYTOTOXIC ASSAYS.—Extracts, fractions, and compounds were tested for cytotoxic activity with eight cell lines, BC1 (human breast cancer), HT-1080 (human fibrosarcoma), Lu1 (human lung cancer), Me12 (human melanoma), Co12 (human colon cancer), KB (human nasopharyngeal carcinoma), KB-V (vincristine-resistant KB), and P-388 (murine lymphocytic leukemia), by the procedures basically established by the National Cancer Institute (32) as described previously (33–35). The test was duplicated at five concentrations (20.0, 4.0, 0.8, 0.16, and 0.032 μg/ml) pure compounds.

PLANT MATERIAL.—The roots of *M. calabura* were collected for detailed phytochemical investigation by Dr. D.A. Madulid in a second growth forest in Marikina (Manila), Philippines, in June 1989. The identity of the sample was confirmed by a taxonomist (D.D.S.). Herbarium specimens representing this collection are deposited in the Philippine National Herbarium, Manila, Philippines and at the Herbarium of the Field Museum of Natural History, Chicago. For preliminary screening of cytotoxicity, the roots of *M. calabura* were collected in June 1988 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 plant material (7.3 kg) was exhaustively extracted with MeOH at room temperature to afford 600 g of residue on removal of solvent under vacuum. This extract was taken up in MeOH (200 ml), and the resultant suspension in H_2O (3 liters) was treated with Et_2O (3 × 3 liters). The Et_2O layer was concentrated to dryness, and the residue (65 g) was chromatographed batchwise over Si gel (1.5 kg) with $CHCl_3$ and $CHCl_3$/MeOH as eluent. Fractions of 500 ml each were collected and checked on tlc to produce eight pooled fractions: F1–2 ($CHCl_3$), F3–4 ($CHCl_3$), F5–8 ($CHCl_3$), F9–14 [$CHCl_3$-MeOH (50:1)], F15–20 [$CHCl_3$-MeOH (20:1)], F21–26 [$CHCl_3$-MeOH (10:1)], F27–32 [$CHCl_3$-MeOH (5:1)], and F33–42 [$CHCl_3$-MeOH (3:1)]. F5–8 (14.8 g) and F9–14 (12.5 g) were repeatedly subjected to cc over Si gel by elution with toluene-Me_2CO (40:1 to 10:1), which resulted in the isolation of the following compounds in sequence from the column: (2*S*)-5′-hydroxy-7,3′,4′-trimethoxyflavan [**1**] (350 mg, 0.0048% w/w), (2*S*)-7,8,3′,4′,5′-pentamethoxyflavan [**2**] (600 mg, 0.0082% w/w), (2*S*)-2′-hydroxy-7,8,3′,4′,5′-pentamethoxyflavan [**3**] (1.6 g, 0.0219% w/w), (2*S*)-5′-hydroxy-7,8,3′,4′-tetramethoxyflavan [**4**] (3.3 g, 0.0452% w/w), (2*S*)-8-hydroxy-7,3′,4′,5′-tetramethoxyflavan [**5**] (430 mg, 0.0059% w/w), (2*S*)-8,2′-dihydroxy-7,3′,4′,5′-tetramethoxyflavan [**6**] (2.1 g, 0.0288% w/w), and (2*S*)-8,5′-dihydroxy-7,3′,4′-trimethoxyflavan [**7**] (4.0 g, 0.0548% w/w). Compounds **5** and **7** were finally purified by crystallization from MeOH.

F15–20 (9.2 g) was subjected to chromatographic separation with a series of Si gel columns eluting with toluene-Me_2CO (20:1 to 10:1) and EtOAc, which resulted in the sequential isolation of the following compounds: 7,8,3′,4′,5′-pentamethoxyflavone [**8**] (68 mg, 0.0009% w/w), (*M*),(2*S*),(2″*S*)-,(*P*),(2*S*),(2″*S*)-8,8″-5′-trihydroxy-7,7″-3′,3‴-4′,4‴-5‴-heptamethoxy-5,5″-biflavan [**9**] (106 mg, 0.0015% w/w), 5′-hydroxy-7,8,3′,4′-tetramethoxyflavone [**10**] (200 mg, 0.0027% w/w), and (*M*),(2*S*),(2″*S*)-,(*P*),(2*S*),(2″*S*)-8,8″-5′,5‴-tetrahydroxy-7,7″-3′,3‴-4′,4‴-hexamethoxy-5,5″-biflavan [**11**] (470 mg, 0.0064% w/w). Compounds **8**, **9**, **10**, and **11** were finally purified by crystallization from MeOH.

F21–26 (6.1 g), which was purified by elution with toluene-Me_2CO (7:1) over a Si gel column, afforded 8,5′-dihydroxy-7,3′,4′-trimethoxyflavone [**12**] (170 mg, 0.0023% w/w). Compound **12** was finally purified by crystallization from MeOH.

CHARACTERIZATION OF COMPOUNDS **1–12**.—*(2S)-5′-Hydroxy-7,3′,4′-trimethoxyflavan* [**1**].—Colorless oil: $[\alpha]^{20}D$ −25.0° (*c* = 0.22, $CHCl_3$); uv λ max (MeOH) (log ε) 227 (4.11), 280 (3.61), 288 (sh) (3.51) nm; cd (MeOH) (Δε) 296 (0), 284 (−1.3), 265 (0); ir ν max (KBr) 3430, 2930, 1620, 1600, 1510, 1470, 1200, 1160, 1110 cm^{-1}; ^1H nmr see Table 2; ^{13}C nmr see Table 3; eims *m/z* (rel. int.) $[M]^+$ 316 (100), 180 (76), 167 (37), 165 (47), 137 (48); hreims *m/z* $[M]^+$ 316.1309 (calcd for $C_{18}H_{20}O_5$, 316.1311).

(2S)-7,8,3′,4′,5′-Pentamethoxyflavan [**2**].—Colorless oil: $[\alpha]^{20}D$ −75.0° (*c* = 0.30, $CHCl_3$); uv λ max (MeOH) (log ε) 229 (4.18), 274 (3.37) nm; cd (MeOH) (Δε) 295 (0), 283 (−1.0), 257 (−0.2); ir ν max (KBr) 2940, 1590, 1500, 1470, 1420, 1240, 1130, 1110, 1080 cm^{-1}; ^1H nmr see Table 2; ^{13}C nmr see Table 3; eims *m/z* (rel. int.) $[M]^+$ 360 (100), 329 (8), 194 (91), 181 (26), 179 (39), 151 (20); hreims *m/z* $[M]^+$ 360.1563 (calcd for $C_{20}H_{24}O_6$, 360.1573).

(2S)-2′-Hydroxy-7,8,3′,4′,5′-pentamethoxyflavan [**3**].—Colorless oil; $[\alpha]^{20}D$ −15.4° (*c* = 0.74, $CHCl_3$); uv λ max (MeOH) (log ε) 227 (4.20), 284 (3.80), 289 (sh) (3.74) nm; cd (MeOH) (Δε) 290 (0), 285 (−0.3), 264 (0); ir ν max (KBr) 3430, 2930, 1600, 1500, 1470, 1420, 1200, 1130, 1110, 1080 cm^{-1}; ^1H nmr see Table 2; ^{13}C nmr see Table 3; eims *m/z* (rel. int.) $[M]^+$ 376 (65), 210 (87), 197 (14), 167 (100), 85 (36), 83 (55); hreims *m/z* $[M]^+$ 376.1526 (calcd for $C_{20}H_{24}O_7$, 376.1522).

(2S)-5′-Hydroxy-7,8,3′,4′-tetramethoxyflavan [**4**].—Colorless oil; $[\alpha]^{20}D$ −59.2° (*c* = 0.26, $CHCl_3$); uv λ max (MeOH) (log ε) 229 (4.16), 274 (3.36) nm; cd (MeOH) (Δε) 295 (0), 284 (−1.0), 275 (−0.6), 257 (−0.1); ir ν max (KBr) 3410, 2920, 1600, 1500, 1470, 1230, 1110 cm^{-1}; ^1H nmr see Table 2; ^{13}C nmr see Table 3; eims *m/z* (rel. int.) $[M]^+$ 346 (97), 315 (10), 193 (21), 192 (22), 180 (93), 167 (100), 166 (50), 151 (30); hreims *m/z* $[M]^+$ 346.1408 (calcd for $C_{19}H_{22}O_6$, 346.1416).

(2S)-8-Hydroxy-7,3′,4′,5′-tetramethoxyflavan [**5**].—Mp 154° (colorless prisms, MeOH); $[\alpha]^{20}D$ −48.5° (*c* = 0.38, $CHCl_3$); uv λ max ($CHCl_3$) (log ε) 246 (3.80), 271 (3.26), 279 (sh) (3.11) nm; cd (MeOH) (Δε) 292 (0), 282 (−0.3), 276 (−0.1); ir ν max (KBr) 3430, 2930, 1620, 1590, 1250, 1220 cm^{-1}; ^1H nmr see Table 2; ^{13}C nmr see Table 3; eims *m/z* (rel. int.) $[M]^+$ 346 (70), 194 (100), 181 (25), 179 (36), 152 (9); hreims *m/z* $[M]^+$ 346.1406 (calcd for $C_{19}H_{22}O_6$, 346.1416).

(2S)-8,2′-Dihydroxy-7,3′,4′,5′-tetramethoxyflavan [**6**].—Colorless oil: $[\alpha]^{20}D$ −7.3° (*c* = 0.55, $CHCl_3$); uv λ max (MeOH) (log ε) 226 (4.18), 286 (3.66) nm; cd (MeOH) (Δε) 310 (0), 285 (−0.2), 265 (0); ir ν max (KBr) 3440, 2920, 1500, 1470, 1370, 1200, 1130, 1100, 1080 cm^{-1}; ^1H nmr see Table 2; ^{13}C nmr see Table 3; eims *m/z* (rel. int.) $[M]^+$ 362 (52), 210 (100), 197 (26), 181 (11), 153 (49); hreims *m/z* $[M]^+$ 362.1364 (calcd for $C_{19}H_{22}O_7$, 362.1366).

(2S)-8,5′-Dihydroxy-7,3′,4′-trimethoxyflavan [**7**].—Mp 153° (colorless needles, MeOH); $[\alpha]^{20}D$

TABLE 2. ^{1}H-nmr Spectra of Flavans **1–7** and Flavones **8**, **10**, and **12**.[a]

Proton	Compound									
	1[b]	**2**[b]	**3**[b]	**4**[b]	**5**[b]	**6**[b]	**7**[b]	**8**[b]	**10**[c]	**12**[d]
H-2	4.90 dd (10.2, 2.4)	5.05 dd (9.8, 2.3)	5.40 dd (10.1, 2.1)	4.99 dd (9.5, 2.5)	4.96 dd (9.7, 2.6)	5.41 dd (10.0, 2.1)	4.98 dd (9.7, 2.8)	—	—	—
H-3	2.03(ax) m 2.15(eq) m	2.03(ax) m 2.22(eq) m	1.98(ax) m 2.29(eq) m	1.99(ax) m 2.17(eq) m	2.05(ax) m 2.16(eq) m	2.02(ax) m 2.20(eq) m	2.05(ax) m 2.17(eq) m	6.72 s	6.72 s	6.69 s
H-4	2.89(ax) ddd (16.2, 11.4, 5.9) 2.71(eq) ddd (16.0, 5.1, 3.3)	2.89(ax) ddd (16.4, 10.8, 5.6) 2.71(eq) ddd (16.1, 4.1, 3.8)	2.96(ax) ddd (16.5, 11.4, 5.6) 2.75(eq) ddd (16.1, 4.0, 3.9)	2.88(ax) ddd (16.1, 9.7, 5.5) 2.71(eq) ddd (16.1, 4.6, 4.6)	2.91(ax) ddd (16.6, 11.0, 6.1) 2.75(eq) ddd (16.3, 4.2, 3.6)	2.93(ax) ddd (16.0, 9.8, 5.5) 2.72(eq) ddd (16.0, 4.6, 3.6)	2.92(ax) ddd (16.3, 10.7, 5.7) 2.76(eq) ddd (16.3, 4.3, 3.8)	—	—	—
H-5	6.96 d (9.1)	6.77 d (8.7)	6.78 d (7.4)	6.74 d (8.5)	6.57 d (8.5)	6.57 d (8.7)	6.58 d (8.5)	7.94 d (8.8)	7.92 d (8.9)	7.66 d (8.9)
H-6	6.46 dd (9.0, 2.5)	6.50 d (8.5)	6.50 d (8.6)	6.48 d (8.5)	6.48 d (8.4)	6.48 d (8.5)	6.50 d (8.5)	7.06 d (8.8)	7.10 d (9.0)	7.11 d (8.9)
H-8	6.48 d (2.5)	—	—	—	—	—	—	—	—	—
H-2′	6.56 d (1.8)	6.68 s	—	6.59 d (1.8)	6.65 s	—	6.56 d (2.0)	7.20 s	7.10 d (1.7)	7.17 d (2.1)
H-6′	6.66 d (1.7)	6.68 s	6.80 s	6.65 d (1.9)	6.65 s	6.75 s	6.66 d (1.8)	7.20 s	7.23 d (1.9)	7.25 d (2.1)
7-OMe	3.74 s	3.82 s	3.86 s	3.83 s	3.84 s	3.84 s	3.86 s	4.01 s	4.03 s	4.03 s
8-OMe	—	3.89 s	3.89 s	3.88 s	—	—	—	4.05 s	4.06 s	—
3′-OMe	3.86 s	3.84 s	3.97 s	3.86 s	3.84 s	3.92 s	3.87 s	3.97 s	3.97 s	3.97 s
4′-OMe	3.87 s	3.84 s	3.89 s	3.83 s	3.84 s	3.88 s	3.89 s	3.94 s	3.95 s	3.92 s
5′-OMe	—	3.84 s	3.79 s	—	3.84 s	3.77 s	—	3.97 s	—	—
8-OH	—	—	—	—	5.64 s	5.70 s	5.49 s	—	—	4.70 s
2′-OH	—	—	5.75 s	—	—	6.00 s	—	—	—	—
5′-OH	6.10 s	—	—	6.19 s	—	—	5.88 s	—	4.41 s	4.70 s

[a]Measured at 300 MHz, $\delta_{TMS} = 0$; coupling constants are provided in parentheses.
[b]Obtained in $CDCl_3$.
[c]Obtained in $CDCl_3$-CD_3OD (5:1).
[d]Obtained in $CDCl_3$-CD_3OD (1:1).

TABLE 3. ^{13}C-nmr Spectra of Flavans **1–7** and Flavones **8**, **10**, and **12**.[a]

Carbon	Compound									
	1[b]	**2**[b]	**3**[b]	**4**[b]	**5**[b]	**6**[b]	**7**[b]	**8**[b]	**10**[c]	**12**[d]
C-2	77.7 d	77.6 d	73.3 d	77.0 d	78.2 d	73.0 d	78.3 d	162.5 s	163.4 s	164.9 s
C-3	29.9 t	29.9 t	28.2 t	29.4 t	29.8 t	28.3 t	30.0 t	106.4 d	105.6 d	106.0 d
C-4	24.3 t	24.3 t	24.6 t	23.9 t	24.0 t	24.2 t	24.3 t	177.9 s	178.7 s	180.3 s
C-5	129.8 d	123.3 d	123.4 d	123.0 d	118.5 d	118.6 d	118.9 d	120.9 d	120.7 d	116.1 d
C-6	107.3 d	104.3 d	104.4 d	104.1 d	104.2 d	104.2 d	104.5 d	109.9 d	110.0 d	110.2 d
C-7	158.9 s	151.6 s	151.6 s	151.2 s	145.3 s	145.3 s	145.6 s	156.6 s	156.7 s	152.4 s
C-8	101.5 d	137.2 s	137.3 s	136.9 s	133.7 s	133.8 s	134.0 s	136.8 s	136.5 s	135.6 s
C-9	155.5 s	148.5 s	148.8 s	148.2 s	142.3 s	142.6 s	142.6 s	150.4 s	150.3 s	146.7 s
C-10	113.8 s	115.7 s	116.2 s	115.5 s	115.1 s	115.5 s	115.4 s	118.6 s	117.9 s	118.6 s
C-1′	137.7 s	137.4 s	121.8 s	137.5 s	136.7 s	121.3 s	137.5 s	126.9 s	126.6 s	127.7 s
C-2′	101.8 d	102.8 d	140.0 s	101.4 d	102.9 d	139.9 s	102.1 d	103.5 d	101.8 d	102.9 d
C-3′	152.4 s	153.1 s	140.1 s	152.0 s	152.9 s	140.0 s	152.5 s	153.5 s	153.1 s	154.2 s
C-4′	135.0 s	137.2 s	141.3 s	134.6 s	137.3 s	141.4 s	135.1 s	141.1 s	139.4 s	140.4 s
C-5′	149.2 s	153.1 s	146.5 s	148.9 s	152.9 s	146.4 s	149.3 s	153.5 s	150.4 s	151.4 s
C-6′	106.0 d	102.8 d	104.9 d	105.3 d	102.9 d	105.0 d	106.0 d	103.5 d	107.4 d	108.6 d
7-OMe	55.1 q	56.0 q	56.2 q	55.8 q	56.0 q	56.1 q	56.4 q	56.4 q	56.2 q	56.8 q
8-OMe	—	60.6 q	60.8 q	60.3 q	—	—	—	61.3 q	61.3 q	—
3′-OMe	55.7 q	55.9 q	61.2 q	55.4 q	55.7 q	61.0 q	55.9 q	56.1 q	55.7 q	56.5 q
4′-OMe	60.7 q	60.7 q	60.9 q	60.4 q	60.4 q	60.6 q	60.9 q	60.9 q	60.5 q	61.0 q
5′-OMe	—	55.9 q	56.4 q	—	55.7 q	56.3 q	—	56.1 q	—	—

[a]Measured at 75.6 MHz, $\delta_{TMS} = 0$.
[b]Obtained in $CDCl_3$.
[c]Obtained in $CDCl_3$-CD_3OD (5:1).
[d]Obtained in $CDCl_3$-CD_3OD (1:1).

$-45.5°$ ($c = 0.20$, $CHCl_3$); uv λ max ($CHCl_3$ (log ε) 245 (3.72), 270 (3.26), 278 (sh) (3.15) nm; cd (MeOH) (Δε) 291 (0), 283 (−0.3), 275 (−0.2), 267 (−0.1); ir ν max (KBr) 3360, 2930, 1610, 1590, 1460, 1220, 1100 cm^{-1}; ^{1}H nmr see Table 2; ^{13}C nmr see Table 3; eims *m/z* (rel. int.) $[M]^+$ 332 (52), 180 (100), 167 (25), 165 (39), 153 (20), 152 (21); hreims *m/z* $[M]^+$ 332.1265 (calcd for $C_{18}H_{20}O_6$, 332.1260).

7,8,3′,4′,5′-Pentamethoxyflavone [**8**].—Mp 206° (colorless prisms, MeOH); uv λ max ($CHCl_3$) (log ε) 241 (4.34), 262 (sh) (4.11), 316 (4.36) nm; ir ν max (KBr) 3280, 2930, 1640, 1600, 1500, 1290, 1120 cm^{-1}; ^{1}H nmr see Table 2; ^{13}C nmr see Table 3; eims *m/z* (rel. int.) $[M]^+$ 372 (100), 357 (15), 329 (4), 192 (9), 172 (9), 152 (12); hreims *m/z* $[M]^+$ 372.1206 (calcd for $C_{20}H_{20}O_7$, 372.1209).

TABLE 4. ^{1}H-nmr Spectra of Biflavans **9** and **11**.[a]

Proton	Compound			
	9		**11**	
	M[b]	*P*[b]	*M*[b]	*P*[b]
H-2	4.90 d (9.4)	4.90 d (9.4)	4.90 d (9.1)	4.90 d (9.1)
H-3	1.70-2.13 m	1.70-2.13 m	1.70-2.12 m	1.70-2.12 m
H-4	2.25-2.72 m	2.25-2.72 m	2.25-2.70 m	2.25-2.70 m
H-6	6.38 s	6.30 s	6.36 s	6.30 s
H-2′	6.59 d (2.1)	6.59 d (2.1)	6.58 d (2.3)	6.58 d (2.3)
H-6′	6.61 s	6.61 s	6.61 s	6.61 s
7-OMe	3.75 s	3.74 s	3.75 s	3.74 s
3′-OMe	3.77 s	3.79 s	3.77 s	3.78 s
4′-OMe	3.66 s	3.68 s	3.66 s	3.68 s
8-OH	8.13 s	8.13 s	8.11 s	8.11 s
5′-OH	9.17 s	9.20 s	9.17 s	9.20 s
H-2″	4.97 d (9.9)	4.97 d (9.9)	4.90 d (9.1)	4.90 d (9.1)
H-3″	1.70-2.13 m	1.70-2.13 m	1.70-2.12 m	1.70-2.12 m

TABLE 4. Continued.

Proton	Compound			
	9		11	
	M[b]	*P*[b]	*M*[b]	*P*[b]
H-4″	2.25-2.72 m	2.25-2.72 m	2.25-2.70 m	2.25-2.70 m
H-6″	6.38 s	6.32 s	6.36 s	6.30 s
H-2‴	6.79 s	6.82 s	6.58 d (2.3)	6.58 d (2.3)
H-6‴	6.79 s	6.82 s	6.61 s	6.61 s
7″-OMe	3.75 s	3.74 s	3.75 s	3.74 s
3‴-OMe	3.77 s	3.79 s	3.77 s	3.78 s
4‴-OMe	3.66 s	3.68 s	3.66 s	3.68 s
5‴-OMe	3.81 s	3.79 s	—	—
8″-OH	8.13 s	8.13 s	8.11 s	8.11 s
5‴-OH	—	—	9.17 s	9.20 s

[a]Measured in DMSO-d_6 at 300 MHz, $\delta_{TMS} = 0$; coupling constants are provided in parentheses.
[b]Assignments of *M* and *P* may be reversed. For the sake of convenience, the *M* companion peaks were assigned as those of greater intensity.

(M),(2S),(2″S)-,(P),(2S),(2″S)-*8,8″-5′-Trihydroxy-7,7″-3′,3‴-4′,4‴-5‴-heptamethoxy-5,5″-biflavan* [**9**].—Mp >228° (dec) (colorless prisms, MeOH); $[\alpha]^{22}D + 11.3°$ ($c = 0.31$, $CHCl_3$); uv λ max ($CHCl_3$) (log ε) 247 (4.19), 269 (3.85) nm; cd ($CHCl_3$) (Δε) 305 (0), 289 (−2.3), 275 (−0.2); ir ν max (KBr) 3440, 2930, 1590, 1490, 1450, 1340, 1230, 1130 cm^{-1}; ^1H nmr see Table 4; ^{13}C nmr see Table 5; eims *m/z* (rel. int.) $[M]^+$ 676 (16), 496 (2), 482 (2), 302 (41), 207 (29), 193 (26), 181 (100), 167 (87); hreims *m/z* $[M]^+$ 676.2520 (calcd for $C_{37}H_{40}O_{12}$, 676.2520).

TABLE 5. ^{13}C-nmr Spectra of Biflavans **9** and **11**.[a]

Carbon	Compound			
	9		11	
	M[b]	*P*[b]	*M*[b]	*P*[b]
C-2 (C-2″)	76.8 (77.0)	76.9 (77.1)	76.8 (76.8)	76.9 (76.9)
C-3 (C-3″)	29.7 (29.7)	29.5 (29.5)	29.7 (29.7)	29.5 (29.5)
C-4 (C-4″)	23.3 (23.5)	22.7 (22.9)	23.3 (23.3)	22.7 (22.7)
C-5 (C-5″)	130.0 (130.1)	130.5 (130.6)	130.0 (130.0)	130.5 (130.5)
C-6 (C-6″)	106.2 (106.4)	105.7 (105.9)	106.2 (106.2)	105.7 (105.7)
C-7 (C-7″)	145.9 (145.9)	145.9 (145.9)	145.8 (145.8)	145.9 (145.9)
C-8 (C-8″)	133.7 (133.7)	133.7 (133.7)	133.6 (133.6)	133.7 (133.7)
C-9 (C-9″)	143.7 (143.7)	143.6 (143.6)	143.7 (143.7)	143.6 (143.6)
C-10 (C-10″)	113.7 (113.7)	114.2 (114.2)	113.7 (113.7)	114.2 (114.2)
C-1′ (C-1‴)	137.2 (137.4)	137.2 (137.4)	137.2 (137.2)	137.2 (137.2)
C-2′ (C-2‴)	101.6 (103.7)	101.7 (103.8)	101.6 (101.6)	101.7 (101.7)
C-3′ (C-3‴)	153.0 (152.8)	153.0 (152.8)	153.0 (153.0)	153.0 (153.0)
C-4′ (C-4‴)	135.7 (136.9)	135.8 (137.0)	135.7 (135.7)	135.8 (135.8)
C-5′ (C-5‴)	150.3 (152.8)	150.4 (152.8)	150.3 (150.3)	150.4 (150.4)
C-6′ (C-6‴)	107.2 (103.7)	107.2 (103.8)	107.2 (107.2)	107.2 (107.2)
7-OMe (7″-OMe)	56.1 (56.1)	56.1 (56.1)	56.1 (56.1)	56.1 (56.1)
3′-OMe (3‴-OMe)	55.7 (55.9)	55.7 (55.9)	55.7 (55.7)	55.7 (55.7)
4′-OMe (4‴-OMe)	59.9 (60.0)	59.9 (60.0)	59.9 (59.9)	59.9 (59.9)
5′-OMe (5‴-OMe)	— (55.9)	— (55.9)	— (—)	— (—)

[a]Measured in DMSO-d_6 at 75.6 MHz, $\delta_{TMS} = 0$.
[b]Assignments of *M* and *P* may be reversed. For the sake of convenience, the *M* companion peaks were assigned as those of greater intensity.

5'-Hydroxy-7,8,3',4'-tetramethoxyflavone [**10**].—Mp 212–213° (colorless needles, MeOH); uv λ max ($CHCl_3$) (log ε) 242 (4.31), 253 (4.20), 263 (4.18), 312 (4.38) nm; ir ν max (KBr) 3280, 2940, 1650, 1600, 1520, 1430, 1290, 1110 cm^{-1}; ^1H nmr see Table 2; ^{13}C nmr see Table 3; eims *m/z* (rel. int.) $[M]^+$ 358 (100), 343 (19), 315 (20), 181 (24), 165 (34), 152 (31), 137 (32); hreims *m/z* $[M]^+$ 358.1052 (calcd for $C_{19}H_{18}O_7$, 358.1053).

(M),(2S),(2"S)-,(P),(2S),(2"S)-*8,8"-5',5"'-Tetrahydroxy-7,7"-3',3"'-4',4"'-hexamethoxy-5,5"-biflavan* [**11**].—Mp >211° (dec) (colorless prisms, MeOH); $[\alpha]^{22}D$ +18.9° (*c* = 0.22, $CHCl_3$); uv λ max ($CHCl_3$) (log ε) 244 (4.28), 268 (3.84) nm; cd (MeOH) (Δε) 302 (0), 288 (−1.3), 276 (−0.5); ir ν max (KBr) 3430, 2930, 1600, 1450, 1100 cm^{-1}; ^1H nmr see Table 4; ^{13}C nmr see Table 5; eims *m/z* (rel. int.) $[M]^+$ 662 (8), 482 (2), 302 (24), 193 (29), 180 (10), 167 (100); hreims *m/z* $[M]^+$ 662.2356 (calcd for $C_{36}H_{38}O_{12}$, 662.2363).

8,5'-Dihydroxy-7,3',4'-trimethoxyflavone [**12**].—Mp 225–227° (dec) (light yellow prisms, MeOH); uv λ max ($CHCl_3$) (log ε) 242 (4.22), 259 (sh) (4.20), 269 (4.28), 310 (4.28) nm; ir ν max (KBr) 3440, 2940, 1630, 1570, 1430, 1280, 1110 cm^{-1}; ^1H nmr see Table 2; ^{13}C nmr see Table 3; eims *m/z* (rel. int.) $[M]^+$ 344 (100), 329 (30), 301 (13), 167 (41), 148 (26), 137 (20), 120 (39); hreims *m/z* $[M]^+$ 344.0891 (calcd for $C_{18}H_{16}O_7$, 344.0896).

ACKNOWLEDGMENTS

This investigation was funded, in part, by grant CA33047 with the National Cancer Institute, National Institutes of Health, Bethesda, Maryland. Dr. Domingo A. Madulid, of the Philippine National Herbarium, Manila, Philippines, is thanked for providing plant material used in this investigation. We acknowledge the Nuclear Magnetic Resonance and Mass Spectrometry Laboratories of the Research Resources Center, University of Illinois at Chicago, for expert assistance and for the provision of spectroscopic equipment used in this study. We thank Mr. R.B. Dvorak, of the Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, for recording the high resolution ei mass spectral data.

LITERATURE CITED

1. Y.-H. Choi, J.M. Pezzuto, A.D. Kinghorn, and N.R. Farnsworth, *Planta Med.*, **54**, 511 (1988).
2. B. Menaut, *Bulletin Economique de Indochine*, **32**, 218 (1929).
3. A. Petelot, *Archives de Recherches Agronomique de Cambodge, Laos, Vietnam*, **1**, 130 (1952).
4. L.M. Perry and J. Metzger, "Medicinal Plants of East and Southeast Asia," MIT Press, Cambridge, Massachusetts, 1980, p. 132.
5. L.M. Guerrero, *Philippine Bureau of Forestry Bulletin*, **22**, 207 (1921).
6. E. Quisumbing, *Department of Agriculture and Natural Resources, Technical Bulletin*, **16**, 569 (1951).
7. J. Diguangco, "Notes on Philippine Medicinal Plants," Manila, Philippines, 1959, p. 69.
8. M. Martinez, "Las Plantas Medicinales de México," 4th ed., Ediciones Botas, Mexico City, Mexico, 1959, p. 391.
9. E. Perez-Arbelaez, "Plantas Medicinales y Venenosas de Colombia," Hernando Salazar, Medellin, Colombia, 1975, p. 192.
10. J.T. Roig y Mesa, "Plantas Medicinales, Aromaticas o Venenosas de Cuba," Ministerio de Agricultura, Havana, Cuba, 1945, p. 212.
11. C.D. Daulatabad, R.F. Ankalgi, and J.S. Kulkarni, *J. Food Sci. Technol.*, **19**, 110 (1982).
12. N.A.M. Saleh, A.E.A. El Sherbeiny, and H.I. El Sissi, *Qual. Plant. Mater. Veg.*, **17**, 384 (1969).
13. P.K. Agrawal and R.P. Rastogi, *Heterocycles*, **16**, 2181 (1981).
14. E. Wenkert, H.E. Gottlieb, O.R. Gottlieb, M.O. da S. Pereira, and M.D. Formiga, *Phytochemistry*, **15**, 1547 (1976).
15. K.S. Saini and S. Ghosal, *Phytochemistry*, **23**, 2415 (1984).
16. D.F. Ewing, *Org. Magn. Reson.*, **12**, 499 (1979).
17. R. Sahai, S.K. Agarwal, and R.P. Rastogi, *Phytochemistry*, **19**, 1560 (1980).
18. A. Bax, *J. Magn. Reson.*, **57**, 314 (1984).
19. J.W. Clark-Lewis, L.M. Jackman, and T.M. Spotswood, *Aust. J. Chem.*, **17**, 632 (1964).
20. A. Numata, T. Takemura, H. Ohbayashi, T. Katsuno, K. Yamamoto, K. Sato, and S. Kobayashi, *Chem. Pharm. Bull.*, **31**, 2146 (1983).
21. F. Gao, H. Wang, and T.J. Mabry, *J. Nat. Prod.*, **50**, 23 (1987).
22. G. Cardillo, L. Merlini, and G. Nasini, *J. Chem. Soc. C*, 3967 (1971).
23. L.J. Porter, in "The Flavonoids: Advances in Research since 1980." Ed. by J.B. Harborne, Chapman and Hall, London, 1988, p. 21.
24. H. Achenbach, M. Stocker, and M.A. Constenla, *Phytochemistry*, **27**, 1835 (1988).
25. A.B. Gutierrez and W. Herz, *Phytochemistry*, **27**, 2225 (1988).
26. B. Testa, "Principles of Organic Stereochemistry," Marcel Dekker, New York, 1979, p. 62.

27. G.G. Lyle, *J. Org. Chem.*, **41**, 850 (1976).
28. S.M. Kupchan, C.W. Sigel, R.J. Hemingway, J.R. Knox, and M.S. Udayamurthy, *Tetrahedron*, **25**, 1603 (1969).
29. P.R. Jefferies, J.R. Knox, K.R. Price, and B. Scaf, *Aust. J. Chem.*, **27**, 221 (1974).
30. S.M. Kupchan, C.W. Sigel, J.R. Knox, and M.S. Udayamurthy, *J. Org. Chem.*, **34**, 1460 (1969).
31. J.M Edwards, R.F. Raffauf, and P.W. LeQuesne, *J. Nat. Prod.*, **42**, 85 (1979).
32. R.I. Geran, N.H. Greenberg, M.M. McDonald, A.M. Schumacher, and B.J. Abbott, *Cancer Chemother. Rep.*, **3** (3), 1 (1972).
33. M. Arisawa, J.M. Pezzuto, C. Bevelle, and G.A. Cordell, *J. Nat. Prod.*, **47**, 453 (1984).
34. H. Jayasuriya, J.D. McChesney, S.M. Swanson, and J.M. Pezzuto, *J. Nat. Prod.*, **52**, 325 (1989).
35. G. Blaskó, H.L. Shieh, J.M. Pezzuto, and G.A. Cordell, *J. Nat. Prod.*, **52**, 1363 (1989).

Received 9 July 1990