Phenolic Constituents in *Erythrina* x *bidwilli* and Their Activity against Oral Microbial Organisms

Munekazu IINUMA,*[,a] Toshiyuki TANAKA,[a] Mizuo MIZUNO,[a] Hirobumi YAMAMOTO,[a] Yasuko KOBAYASHI,[b] and Shigetomo YONEMORI[c]

Department of Pharmacognosy, Gifu Pharmaceutical University,[a] 6–1 Mitahora-higashi 5 chome, 502 Gifu, Japan, Department of Microbiology, Aichi-Gakuin University,[b] School of Dentistry, 1–100 Kusumoto-cho, Tikusa-ku, 464 Nagoya, Japan, and Research Institute of Tropical Agriculture, College of Agriculture, The University of Ryukyu,[c] 870 Uehara, Taketomi-cho, 907–15 Okinawa, Japan. *Received March 18, 1992*

Five flavonoid compounds, including two new isoflavanones, were isolated from the root bark of *Erythrina* x *bidwilli*. Their structures were determined to be 2,10-di(γ,γ-dimethylallyl)-3,9-dihydroxypterocarpan (erythrabyssin II), 6,8-di(γ,γ-dimethylallyl)-7,2′,4′-trihydroxyisoflavanone (bidwillon A), 8-γ,γ-dimethylallyl-2′,4′-dihydroxy-[6″,6″-dimethylpyrano-(2″,3″ : 7,6)]isoflavanone (bidwillon B), 8-γ,γ-dimethylallyl-7,4′-dihydroxyisoflavone (8-γ,γ-dimethylallyldaidzein), and 8-γ,γ-dimethylallyl-5,2′,4′-trihydroxy-[6″,6″-dimethylpyrano-(2″,3″ : 7,6)]isoflavone (auriculatin), by means of spectroscopic analysis. Some potent activities against oral microbial organisms (*Fusobacterium nucleatum* and *Prevotella intermedia*) were shown in these flavonoid compounds.

Keywords *Erythrina* x *bidwilli*; Leguminosae; isoflavanone; isoflavone; pterocarpan; oral anti-microbial activity

The genus *Erythrina* (Leguminosae) occurs in the tropical and subtropical zones and comprises about 110 species.[1] Some of the species have been used in traditional medicine, and recent studies on the chemical components of some species have established the presence of bioactive alkaloids and many flavonoid compounds.[2-20] In this paper, flavonoids with anti-microbial activity in the root bark of *E.* x *bidwilli* are described.

Materials and Methods

Isolation The dried root bark (200 g) of *Erythrina* x *bidwilli* collected at Iriomote, Okinawa, Japan, in August, 1989 was pulverized and extracted with acetone (1 l). The acetone solution was concentrated *in vacuo* to give an extract (12 g) which was subjected to silica gel column chromatography eluted with *n*-hexane–acetone (3 : 1), and 300 ml fractions were collected. The fractions were monitored for their anti-microbial activity using eleven bacteria, and the results of the sensitivity test are shown in Table I. *Prevotella intermedia* was sensitive to fractions 6—8 and 10 (6.25 μg/ml) and *Lactobacillus fermentium* and *Streptococcus mutans* OZ1 were also sensitive to fraction 6 (6.3 μg/ml). After combination, purification of fractions 2—13 by silica gel chromatography, preparative thin layer chromatography (TLC) and recrystallization afforded **1** (22 mg), **2** (40 mg), **3** (8 mg), **4** (5 mg) and **5** (4 mg).

Compound 1 A white amorphous powder. Electron ionization mass spectrometry (EIMS) *m/z* (rel. int.): 392 (100), 336 (47), 281 (47), 161 (22), 147 (18). Proton nuclear magnetic resonance (^{1}H-NMR) (270 MHz, $CDCl_3$) δ: 1.74 (3H, s, Me), 1.78 (6H, s, Me × 2), 1.79 (3H, s, Me), 3.31, 3.33 (2H each, d, J = 7 Hz, CH_2), 3.45 (1H, m, H-6a), 3.59 (1H, m, H-6), 4.20 (1H, dd, J = 10, 4 Hz, H-6), 5.29—5.33 (2H, m, CH = × 2), 5.40 (3H, m, H-11a and OH × 2), 6.35 (1H, d, J = 9 Hz, H-8), 6.39 (1H, s, H-4), 6.95 (1H, d, J = 9 Hz, H-7), 7.24 (1H, s, H-1).

Compound 2 (Bidwillon A) A colorless oil. Gibbs reaction (+). $[\alpha]_D^{23}$ −35.3° (c = 1.1, MeOH). EIMS *m/z* (rel. int.): 408 (78), 390 (13), 273 (100), 272 (100), 257 (18), 229 (20), 217 (60), 118 (51), 173 (22), 151 (62), 136 (20). ^{1}H-NMR (270 MHz, acetone-d_6) δ: 1.66 (3H, br s, Me, H-4‴), 1.70 (3H, br s, Me, H-5″), 1.74 (3H, br s, Me, H-5‴), 1.77 (3H, br s, Me, H-4″), 3.27 (2H, d, J = 7 Hz, CH_2, H-1″), 3.38 (2H, d, J = 7 Hz, CH_2, H-1‴), 4.07 (1H, dd, J = 9, 6 Hz, H-3), 4.52 (1H, dd, J = 11, 6 Hz, H-2eq), 4.65 (1H, dd, J = 11, 9 Hz, H-2ax), 5.12 (1H, m, CH =, H-2‴), 5.35 (1H, m, CH =, H-2″), 6.28 (1H, dd, J = 8, 2 Hz, H-5′), 6.42 (1H, d, J = 2 Hz, H-3′), 6.95 (1H, d, J = 8 Hz, H-6′), 7.53 (1H, s, H-5), 8.10, 8.26 (1H each, br s, OH-7, 4′), 8.60 (1H, br s, OH-2′). ^{13}C-NMR (67.5 MHz, acetone-d_6) δ: [all carbons were assigned with the aid of C–H, long-range C–H correlation spectroscopy (COSY), and insensitive nuclei enhanced by polarization transfer (INEPT) methods] 72.1 (t, C-2), 48.0 (d, C-3), 193.0 (s, C-4), 126.4 (d, C-5), 115.9 (s, C-6), 160.3 (s, C-7), 116.8 (s, C-8), 160.9 (s, C-9),

TABLE I. Activity of Each Fraction in Acetone Extract of *Erythrina* x *bidwilli* against Oral Microorganisms

Fraction No.	Microorganisms[a] (MIC, μg/ml)										
	1	2	3	4	5	6	7	8	9	10	11
1	100	>100	25	>100	>100	>100	>100	>100	>100	>100	>100
2	12.5	12.5	25	25	>100	50	12.5	100	>100	100	>100
3	50	25	25	100	100	50	50	100	>100	100	>100
4	12.5	12.5	12.5	25	100	100	12.5	100	25	100	>100
5	25	25	12.5	25	>100	25	12.5	100	50	100	>100
6	12.5	6.3	6.3	25	>100	50	6.25	100	>100	100	>100
7	25	12.5	12.5	12.5	>100	50	6.25	100	>100	50	>100
8	25	25	12.5	25	>100	50	6.25	100	>100	50	>100
9	12.5	12.5	12.5	25	50	25	12.5	100	50	50	>100
10	25	25	12.5	25	100	100	6.25	100	50	>100	>100
11	50	50	50	25	100	100	12.5	100	100	100	>100
12	25	50	50	25	100	100	12.5	100	100	100	>100
13	12.5	25	12.5	25	100	>100	12.5	>100	50	100	>100
14	100	100	100	50	100	100	12.5	100	100	100	>100
15	100	100	100	25	>100	>100	50	>100	>100	>100	>100
16	>100	>100	100	25	>100	100	100	>100	>100	>100	>100
17	>100	>100	>100	25	>100	>100	>100	>100	>100	>100	>100

a) Microorganisms: 1 = *Lactobacillus casei* ATCC 7469; 2 = *Lactobacillus fermentum* YIT 0082; 3 = *Streptococcus mutans* OZI; 4 = *Streptococcus mutans* Ingbritt; 5 = *Fusobacterium nucleatum* ATCC 10953; 6 = *Porphyromonas gingivaris* CTT 33277; 7 = *Prevotella intermedia* ATCC 26551; 8 = *Actinobacillus actinomycetemcomitans*; 9 = *Actinomyces naeslundii* ATCC 10953; 10 = *Staphylococcus aureus* NIHJ 209P; and 11 = *Escherichia coli* NIHJ K12.

123.6 (s, C-10), 115.3 (s, C-1′), 157.7 (s, C-2′), 104.3 (d, C-3′), 159.1 (s, C-4′), 108.2 (d, C-5′), 131.5 (d, C-6′), 29.2 (t, C-1″), 23.3 (t, C-1‴), 123.2 (d, C-2″), 123.5 (d, C-2‴), 134.2 (s, C-3″), 132.8 (s, C-3‴), 26.3 (q, C-4″, C-4‴), 18.3 (q, C-5″), 18.4 (q, C-5‴).

Compound 3 (Bidwillon B) A colorless amorphous powder. $[\alpha]_D^{22}$ 0° ($c=0.1$, MeOH). EIMS m/z (rel. int.): 406 (67), 391 (100), 373 (56), 271 (42), 255 (22), 241 (22), 237 (23), 205 (36). ^{1}H-NMR (acetone-d_6) δ: 1.45, 1.46 (3H each, s, chromene Me), 1.66, 1.79 (3H each, br s, H-4‴, 5‴), 3.31 (2H, br d, $J=7$ Hz, CH_2, H-1‴), 4.13 (1H, dd, $J=10$, 5 Hz, H-3), 4.60 (1H, dd, $J=11$, 5 Hz, H-2eq), 4.71 (1H, dd, $J=11$, 10 Hz, H-2ax), 5.21 (1H, br t, $J=7$ Hz, CH=, H-2‴), 5.73 (1H, dd, $J=10$, 0.4 Hz, H-5″), 6.31 (1H, dd, $J=8$, 2 Hz, H-5′), 6.43 (1H, d, $J=2$ Hz, H-3′), 6.47 (1H, d, $J=10$ Hz, H-4″), 7.45 (1H, d, $J=0.4$ Hz, H-5), 8.22, 8.53 (1H each, br s, OH).

Compound 4 A pale yellow amorphous powder. EIMS m/z (rel. int.): 322 (82), 307 (29), 267 (100), 149 (24), 118 (13). ^{1}H-NMR (acetone-d_6) δ: 1.69, 1.73 (3H each, br s, Me, H-4″, 5″), 3.57 (2H, d, $J=7$ Hz, CH_2, H-1″), 5.29 (1H, br t, $J=7$ Hz, CH=, H-2″), 6.89 (2H, d, $J=9$ Hz, H-3′, 5′), 7.03 (1H, d, $J=9$ Hz, H-6), 7.48 (2H, d, $J=9$ Hz, H-2′,6′), 7.92 (1H, d, $J=9$ Hz, H-5), 8.24 (1H, s, H-2). ^{13}C-NMR (DMSO-d_6) δ: 152.9 (C-2), 122.6 (C-3), 175.1 (C-4), 124.1 (C-5), 115.0 (C-6), 162.6 (C-7), 114.2 (C-8), 155.2 (C-9), 116.6 (C-10), 123.1 (C-1′), 114.9 (C-2′, 6′), 130.1 (C-3′, 5′), 157.1 (C-4′), 21.6 (C-1″), 122.6 (C-2″), 131.4 (C-3″), 25.5 (C-4″), 17.8 (C-5″).

Compound 5 A yellow amorphous powder. EIMS m/z (rel. int.): 420 (56), 405 (100), 377 (22), 365 (20), 337 (23), 215 (33). ^{1}H-NMR (acetone-d_6) δ: 1.49 (6H, s, Me×2, chromene Me), 1.67, 1.83 (3H each, br s, Me, H-4‴, 5‴), 3.43 (2H, d, $J=7$ Hz, CH_2, H-1‴), 5.22 (1H, br t, $J=7$ Hz, CH=, H-2‴), 5.78 (1H, d, $J=10$ Hz, H-5″), 6.47 (1H, dd, $J=9$, 2 Hz, H-5′), 6.49 (1H, d, $J=2$ Hz, H-3′), 6.69 (1H, d, $J=10$ Hz, H-4″), 7.15 (1H, d, $J=9$ Hz, H-6′), 8.27 (1H, s, H-2), 8.34, 8.48 (1H each, br s, OH-2′, 4′), 13.13 (1H, s, OH-5).

Growth of Bacteria Facultative anaerobes (*Lactobacillus*, *Streptococcus*, *Staphylococcus* and *Escherichia*) were cultivated in Müller-Hinton broth (Difco) containing 0.5% yeast extract and 0.5% glucose in the air at 37 °C for 18—48 h. *Fusobacterium*, *Porphyromonas*, *Prevotella*, *Actinobacillus* and *Actinomyces* were cultivated in Müller-Hinton broth containing 0.5% yeast extract, 0.5% glucose, hemin (5 μg/ml) and menadione (1 μg/ml) in an anaerobic glove box (model AZ: Hirasawa, Tokyo) in the atmosphere of 80% N_2, 10% H_2 and 10% CO_2 at 37 °C for 3—5 d.

Sensitivity Test Minimum inhibitory concentration (MIC) was determined by the agar dilution method essentially as described by the Association of Chemotherapy Japan.[21] Inocula were prepared by dilution of 18 h—15 d broth (10^8—10^9 cells/ml) with buffered saline to 1×10^6 colony forming units/ml. Plates containing 5% (v/v) defibrinated horse blood culture medium (*vide supra*) were inoculated with a loop (i.d. 1 mm). The inoculated plates were incubated at 37 °C for 3—5 d in the air or an anaerobic glove box. The MIC was defined as the lowest concentration of antimicrobial agent in the agar medium resulting in complete inhibition of visible growth.

Results and Discussion

Compound **1** was obtained as a white amorphous powder and gave M^+ at m/z 392 in the EIMS spectrum. In the ^{1}H-NMR spectrum, a set of four-protons (δ 3.45, 3.59, 4.20 and 5.40) assignable to the protons at C-6, C-6a and C-11a suggested that **1** had a peterocarpan skeleton. In the spectrum, the signals due to two γ,γ-dimethylallyl groups [δ 1.74 (Me), 1.78 (Me×2), 1.79 (Me), 3.31, 3.33 (1H each, d, $J=7$ Hz, CH_2), 5.29—5.33 (2H, m, 2×CH=)], and two phenolic hydroxyl protons (δ 5.40 overlapped with H-11a), and two singlets (δ 6.39 and 7.24) and two *ortho*-coupled doublets (δ 6.35 and 6.95) were observed in the aromatic region. These data were identical with those of 2,10-di(γ,γ-dimethylallyl)-3,9-dihydroxypterocarpan (erythrabyssin II) isolated from *E. abyssinica*.[2]

A new compound **2**, called bidwillon A, was obtained as a colorless oil, and gave M^+ at m/z 408. In the ^{1}H-NMR spectrum, a set of one-proton double doublets [δ 4.07 ($J=9$, 6 Hz), 4.52 ($J=11$, 6 Hz) and 4.65 ($J=11$, 9 Hz)] was observed. In the C–H COSY spectrum, the former (δ 4.07) correlated with a carbon at δ 48.0, and the latter two protons caused a cross peak with a carbon at δ 72.1. These results indicated that the protons could be assigned to H-2 and H-3 in an isoflavanone skeleton. In the ^{1}H-NMR spectrum, the presence of two γ,γ-dimethylallyl groups [δ 1.66, 1.70, 1.74, 1.77 (Me), 3.27, 3.38 (2H each, d, $J=7$ Hz), 5.12, 5.35 (1H each, m, CH=)], and three hydroxyl groups (δ 8.10, 8.26, 8.60) was further suggested. In an aromatic region, a singlet proton shielded by a carbonyl group (δ 7.53), and three protons in an ABX spin system [δ 6.28 (dd, $J=8$, 2 Hz), 6.42 (d, $J=2$ Hz) and 6.95 (d, $J=8$ Hz)] were observed. In the ^{13}C-NMR spectrum, two methylene carbons due to a γ,γ-dimethylallyl group were observed at δ 23.3 and 29.2. Based on an systematic study by Fukai *et al.*,[22] the former carbon was assigned to a methylene carbon of the γ,γ-dimethylallyl group *ortho*-substituted with two *O*-functions, and the latter to that of *ortho* located by an *O*-function. These results suggested that the structure for bidwillon A was either **2** or **2a** (Fig. 2). Bidwillon A gave a purple spot on the TLC plate by Gibbs reaction, which suggested that structure **2** was preferable to **2a**. An unambiguous structure confirmation was established by

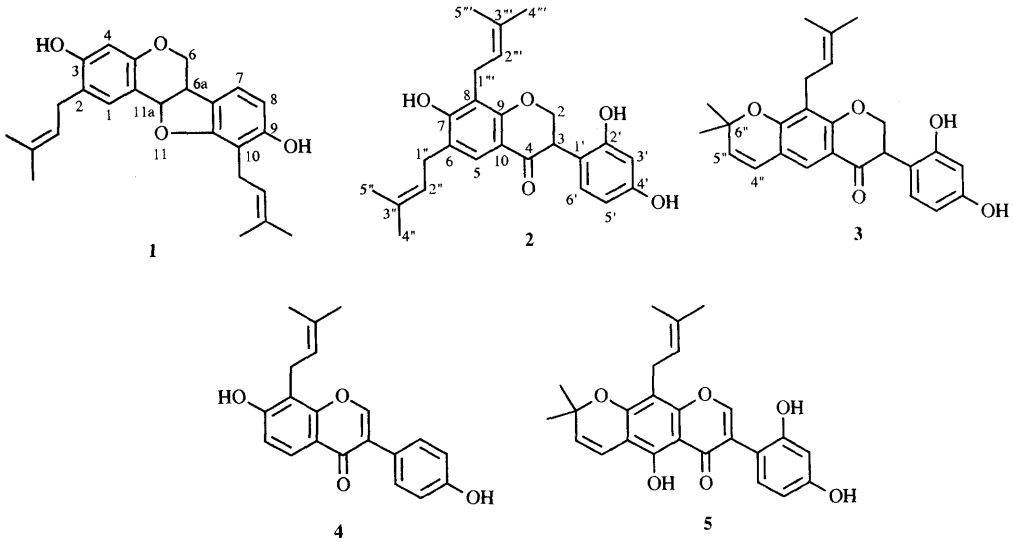
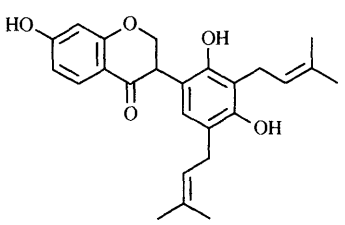


Fig. 1

TABLE II. Activity of the Isolated Compounds (**1**—**5**) against Oral Microorganisms

Compound	Microorganisms[a] (MIC, μg/ml)										
	1	2	3	4	5	6	7	8	9	10	11
1	>50	>50	>50	>50	6.25	50	12.5	>50	50	>50	>50
2	>50	>50	>50	>50	25	50	3.2	12.5	>50	>50	>50
3	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
4	>50	>50	>50	>50	6.25	>50	>50	>50	>50	>50	>50
5	>50	>50	>50	>50	3.2	>50	>50	>50	>50	>50	>50
Erythromycin	0.2	0.4	0.4	0.4	0.4	0.2	0.4	0.4	0.4	6.25	>50
Tetracycline	12.5	12.5	3.2	3.2	12.5	0.2	0.2	12.5	6.26	12.5	25

a) Microorganisms are listed in Table I.



2a

Fig. 2

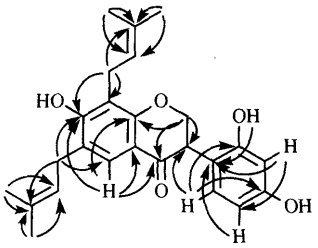


Fig. 3

long-range two-dimensional NMR (2D-NMR). In the spectrum, an aromatic singlet (δ 7.53) caused a cross peak with one of the γ,γ-dimethylallyl methylene protons at δ 3.35, indicating that the γ,γ-dimethylallyl group was adjacent to the aromatic singlet. In the long range C–H COSY spectrum, this singlet caused a cross peak with the carbonyl carbon (δ 193.9) through ^3J, while, on the contrary, an aromatic doublet (δ 6.95) correlated with a carbon at C-3 (Fig. 3), which indicated that the singlet and the doublet protons were assignable to H-5 and H-6′, respectively. Consequently, the structure of bidwillon A was determined to be 6,8-di(γ,γ-dimethylallyl)-7,2′,4′-trihydroxyisoflavanone (**2**).

Another new compound **3**, bidwillon B, was obtained as a colorless amorphous powder, and gave M^+ at m/z 406 in the EIMS spectrum. The ^{1}H-NMR spectrum was almost the same as that of **2**, except for the presence of a dimethylchromene ring, the numeral of γ,γ-dimethylallyl group, and the chemical shift of a proton (δ 7.45) assigned to H-5 which was coupled with one of the chromene protons (δ 5.73) in a doublet (J=0.4 Hz). Accordingly, one of two γ,γ-dimethylallyl groups in **2** was cyclized with 7-OH in **3** to form a chromene ring. In the nuclear Overhauser effect spectroscopy (NOESY) spectrum, an NOE was observed between H-5 and a chromene proton at δ 6.47, which indicated that the chromene ring was attached in a linear form. The structure of bidwillon B was then concluded to be 8-γ,γ-dimethylallyl-2′,4′-dihydroxy-[6″,6″-dimethyl-pyrano(2″,3″ : 7,6)]isoflavanone (**3**).

7,2′,4′-Trioxygenated isoflavanones such as **2** and **3** are so-called homospecific flavonoid compounds,[23] and are a third instance of naturally occurring compounds, in addition to a 2′-oxygenated flavone in *Primula*,[24] and 5,7,2′,4′,6′-pentaoxygenated flavonoids in *Sophora*[25] and *Echinosophora*.[25]

Compounds **4** and **5** gave M^+ at m/z 322 and 420, respectively. In the ^{1}H-NMR spectrum, a typical singlet assigned to H-2 of an isoflavone skeleton was observed at δ 8.24 in **4**, and at δ 8.27 in **5**. The ^{1}H-NMR spectrum of **4** showed the presence of a γ,γ-dimethylallyl group, a pair of *ortho*-coupled one-proton doublets, and a set of two-proton doublets due to 4′-oxygenated isoflavone. Consequently, the structure of **4** was 8-γ,γ-dimethylallyl-7,4′-dihydroxyisoflavone (8-γ,γ-dimethylallyldaidzein), which was recently isolated from *Pueraria lobata*.[26] On the other hand, the ^{1}H-NMR spectrum of **5** exhibited the presence of a γ,γ-dimethylallyl group, a dimethylpyran ring, and three protons in an ABX spin system due to a 2′,4′-oxygenated isoflavone, as well as three hydroxyl groups, including a chelated one. By comparing spectral data with those of an authentic sample, the structure of **5** was concluded to be 8-γ,γ-dimethylallyl-5,2′,4′-trihydroxy-[6″,6″-dimethylpyrano(2″,3″ : 7,6)]isoflavone (auriculatin) isolated from *E. senegalensis*.[10]

The MICs of the five isolated flavonoid compounds against oral microbes are shown in Table II. The activities of these compounds were found to be less than those of the known antibiotics erythromycin or tetracycline, but they showed potent activities against *Fusobacterium nucleatum* in **5**, and *Prevotella intermedia* in **2** (each MIC is 3.2 μg/ml).

The activities of these isolated compounds against *Lactobacillus* and *Streptococcus* were less than those of fractions 2—13. Further characterization of compounds with anti-microbial activity in the root bark of *Erythrina* x *bidwilli*, in particular, to *Lactobacillus* and *Streptococcus* is now in progress.

References

1) H. Ohashi, "Wild Flowers of Japan, Woody Plants I," ed. by Y. Satake, H. Hara, S. Watari, and T. Tominari, Heibonsha, Tokyo, 1989, p. 249.
2) V. S. Kamat, F. Y. Chuo, I. Kubo, and K. Nakanishi, *Heterocycles*, **15**, 1163 (1981).
3) Z. T. Fomum, F. J. Ayafor, and J. T. Mbafor, *Tetrahedron Lett.*,

24, 4127 (1983).
4) L. A. Mitscher, J. A. Ward, S. Drake, and G. S. Rao, *Heterocycles*, **22**, 1673 (1984).
5) Z. T. Fomum, J. F. Ayafor, and J. Wandji, *Phytochemistry*, **24**, 3075 (1984).
6) Z. T. Fomum, J. F. Ayafor, P. N. Ifeadike, A. E. Nkengfack, and J. Wandji, *Planta Medica*, **52**, 341 (1986).
7) Z. T. Fomum, J. F. Ayafor, J. Wandji, W. G. Fomban, and A. E. Nkengfack, *Phytochemistry*, **25**, 757 (1986).
8) R. Promatta, M. S. Tempesta, Z. T. Fomum, J. F. Ayafor, and T. Mbafor, *J. Nat. Prod.*, **49**, 932 (1986).
9) Z. T. Fomum, J. F. Ayafor, J. T. Mbafor, and C. M. Mbi, *J. Chem. Soc., Perkin Trans. 1*, **1986**, 33.
10) R. B. Talor, R. G. Corley, M. S. Tempesta, Z. T. Fomum, J. F. Ayafor, J. Wandji, and P. N. Ifeakike, *J. Nat. Prod.*, **49**, 670 (1986).
11) Z. T. Fomum, J. F. Ayafor, and J. Wandji, *J. Nat. Prod.*, **50**, 921 (1987).
12) L. A. Mitscher, S. Gollapudi, D. C. Gerlach, G. S. Drake, E. A. Beliz, and J. A. Ward, *Phytochemistry*, **27**, 381 (1988).
13) L. A. Mitscher, S. K. Okwute, S. P. Gollapudi, and A. Keshavarz-Shokri, *Heterocycles*, **27**, 2517 (1988).
14) L. A. Mitscher, S. K. Okwute, S. R. Gollapudi, G. S. Drake, and E. Avona, *Phytochemistry*, **27**, 3449 (1988).
15) M. Maillard, M. Hamburger, M. P. Gupta, and K. Hostettmann, *Planta Medica*, **55**, 281.
16) A. E. Knengfack, D. R. Sanson, M. S. Tempesta, and Z. T. Fomum, *J. Nat. Prod.*, **52**, 320 (1989).
17) A. E. Nkengfack, D. R. Sanson, Z. T. Fomum, and M. Tempesta, *Phytochemistry*, **28**, 2522 (1989).
18) H. Telikepalli, S. P. Gollapudi, A. Keshavarz-Shokri, L. Velazquez, R. A. Sandmann, E. A. Veliz, K. V. J. Rao, A. S. Madhavi, and L. A. Mitscher, *Phytochemistry*, **29**, 2005 (1990).
19) J. Wandji, A. E. Nkengfack, Z. T. Fomum, R. Ubillas, B. Killday, and M. S. Tempesta, *J. Nat. Prod.*, **53**, 1425 (1990).
20) A. E. Nkengfack, Z. T. Fomum, R. Ubilla, and M. Tempesta, *J. Nat Prod.*, **53**, 1552 (1990).
21) M. Mitsuhashi, *Chemotherapy*, **29**, 76 (1981).
22) T. Fukai and T. Nomura, *Heterocycles*, **29**, 2379 (1989).
23) M. Iinuma and M. Mizuno, *Phytochemistry*, **28**, 681 (1989).
24) E. Wollenweber, M. Iinuma, T. Tanaka, and M. Mizuno, *Phytochemistry*, **29**, 633 (1990).
25) M. Iinuma, M. Ohyama, T. Tanaka, M. Mizuno, and S.-K. Hong, *Phytochemistry*, **31**, 665 (1992).
26) T. Hakamatsuka, Y. Ebizuka, and U. Sankawa, *Phytochemistry*, **30**, 1481 (1991).