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Michel Sauvain, Jean-Pierre Dedet, Nicole Kunesch, and Jacques Poisson

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ISOLATION OF FLAVANS FROM THE AMAZONIAN SHRUB *FARAMEA GUIANENSIS*

MICHEL SAUVAIN,*

Institut Français de Recherche Scientifique pour le Développement en Coopération (ORSTOM), CP 9214, La Paz, Bolivia

JEAN-PIERRE DEDET,

Laboratoire d'Ecologie Médicale et Pathologie Parasitaire, Annexe de la Faculté de Médecine, 163, rue Auguste Broussonnet 34000, Montpellier, France

NICOLE KUNESCH, and JACQUES POISSON

Laboratoire de Chimie des Substances Thérapeutiques Naturelles, Faculté de Pharmacie, rue J.-B. Clément 92296, Châtenay-Malabry, France

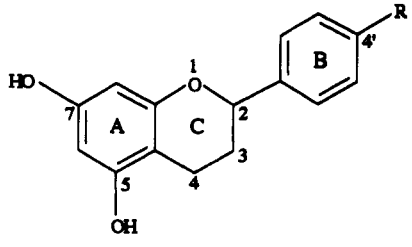
ABSTRACT.—In the pursuit of new leishmanicidal natural products, 5,7,4′-trihydroxyflavan [**1**] and the new product, 5,7-dihydroxy-4′-methoxyflavan [**2**], were isolated from the Guianian medicinal plant *Faramea guianensis*.

A plant screening program for potential leishmanicides was initiated in 1984 in French Guiana (1), and was based on the ethnomedical knowledge of local populations of tropical America and particularly of the table-lands of the Guianas (2). In contrast to certain other diseases, leishmaniasis is properly identified as a disease by the natives from the Amazonian forest (3) and screening for new leads on the grounds of ethnomedicine may be justified.

The leishmanicidal activity of several plant extracts was evaluated in vitro by testing on amastigote stages of *Leishmania amazonensis* and in vivo using cutaneous *L. amazonensis* lesions in mice. This research allowed the prioritization of several species. Among the selected species, *Faramea guianensis* (Aublet) Bremek (Rubiaceae) showed leishmanicidal activity in vitro. This communication describes the isolation and synthesis of active constituents of this shrub.

Bioassay-linked extraction of an extract of *F. guianensis* showed the CH_2Cl_2 and the EtOAc fractions to be the most active. Further purification of these fractions resulted in the isolation of two compounds, **1** and **2**.

The infrared spectrum of the first compound [**1**] revealed the presence of



1 R=OH
2 R=OCH_3

the bands at 3390, 1605, and 800 cm^{-1} characteristic of hydroxyl groups and an aromatic nucleus. The ^{1}H-nmr spectrum was more significant with a double doublet at 4.18 ppm (J=12.5 and 2.5 Hz) coupled with multiplets at 1.83, 2.00 and 2.52 ppm being suggestive of the pyran nucleus of a flavan. Furthermore, this isolate could be identified as a 5,7-dihydroxyflavan through a typical meta-coupled pattern for H-6 and H-8 (doublets at 5.67 and 5.81 ppm, J_{meta}=2.5 Hz) with an additional hydroxyl group in the para position of the B-ring (two doublets at 6.68 and 7.12 ppm, J_{ortho}=9 Hz). The mass spectrum was in agreement with the presence of 5,7,4′-trihydroxyflavan [**1**] (molecular ion peak at *m/z* 258 and characteristic fragments at *m/z* 120 and 107 of a hydroxy-substituted B-ring). The final identification was achieved by

comparison with the synthetic compound obtained in one step by reduction of naringenin (4,5). The compound 5,7,4′-trihydroxyflavan **1** has been isolated for the first time as a natural product. Until now, only the 5-xyloside of **1** has been isolated from the leaves of *Buckleya lanceolata* (6) while the related 5,7-dimethoxy-4′-hydroxyflavan has been isolated from a palm tree (7).

The second compound isolated from *F. guianensis* showed structural similarity with compound **1**. The 5,7,4′-trisubstituted flavan was easily recognized from its ^{1}H-nmr spectrum: a dihydropyran system with protons at 1.96 and 2.10 ppm (H-3), 2.62 ppm (H-4) and 4.87 ppm (J=12.5 and 2.5 Hz; H-2); a disubstituted A-ring with doublets at 5.90 ppm (J_{meta}=2.5 Hz, H-6) and 5.95 ppm (J_{meta}=2.5 Hz, H-8); a monosubstituted B-ring characterized by doublets at 6.83 ppm (J_{ortho}=9 Hz, H-3′, H-5′) and 7.26 ppm (J_{ortho}=9 Hz, H-2′, H-6′). The presence of a methoxy group was revealed by the signals at 3.75 ppm (^{1}H-nmr) and 55.7 ppm (^{13}C-nmr). The characteristic fragments at *m/z* 134 and 121 in the mass spectrum ([M]$^+$ *m/z* 272) were in favor of a methoxy-substituted B-ring. The proposed structure for this new compound is 5,7-dihydroxy-4′-methoxyflavan [**2**].

The two flavans **1** and **2** were isolated from *F. guianensis*. Within the genus *Faramea*, only one other species, *Faramea cyanea*, from the Brazilian Amazonian forest has been investigated and two naphthopyran compounds have been isolated (8).

The lethal action of a crude extract of the leaves of *F. guianensis* against amastigotes in an in vitro macrophage culture system was observed as a gradual decrease in the percentage of infection with the increase of concentration (100–2000 μg/ml)(Table 1). The fraction which contained the mixture of the two flavans showed an interesting activity in vitro with an ED_{50} against amastigotes of 10 μg/ml and no toxicity on macrophages at a fifth of this dose. The synthetic trihydroxyflavan **1** obtained in large amounts showed a weak activity at 60 μg/ml in the amastigote model but was devoid of activity on cutaneous *L. amazonensis* lesions in mice (data not shown). The probable reason for this negative in vivo result is an insufficient con-

TABLE 1. In Vitro Antileishmanial Activity of Fractions and Compounds from *Faramea guianensis*.

Sample	Dose (mg/ml)	Inhibition of infected macrophages (%)	Survival of peritoneal macrophages (%)
Decoction of leaves of *F. guianensis* (JJDG 7489)	2	2	60
	1	29	90
	0.5	55	95
Decoction of leaves of *F. guianensis* (JJDG 8834)	2	—	15
	1	13	95
	0.5	69	100
	0.1	98	100
EtOAc fraction of leaves of *F. guianensis* (JJDG 8834)	2	—	0
	1	0	85
	0.5	38	100
	0.1	87	100
5,7,4-trihydroxyflavan (synthetic) [**1**]	0.25	—	0
	0.05	57	—
	0.04	100	—
	0.02	100	—

centration of the active product at the level of the cutanous lesion.

This type of flavan with various substituents on the aromatic nucleus, particularly amine substituents at C-6, has been previously examined for antiviral properties (9,10).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were measured with a Kofler hot-stage and are uncorrected. The ir spectra were obtained in KBr on a Perkin-Elmer 257 spectrometer. The mass spectra were obtained under electron impact (ei) with Nermag/Sidar V 2.3 and AEI MS-50 instruments. The ^{1}H-nmr spectra were measured on a Bruker AM-200SY spectrometer. Chemical shifts (δ) are expressed in ppm relative to tetramethylsilane (TMS, $\delta=0$). Coupling constants (J) are indicated in Hertz (Hz).

Tlc was carried out using F 1500 LS 254 Si gel plates (Schleicher-Schüll, Germany). These plates were observed in uv light at 254 and 366 nm and sprayed with a 20% H_2SO_4 before heating at 100-110°. Column chromatography used type 60 Si gel (particle size 0.063–0.20 mm). Prep. hplc was performed with a Waters 500 instrument and a type 60 Si gel column (particle size 0.010–0.040 mm) under a pressure of 10 bar.

PLANT MATERIAL.—*Faramea guianensis* (Aublet) Bremek was collected by J.-J. de Granville in French Guiana and herbarium samples were deposited under the numbers de Granville 7489 and 8834 at the Herbarium of the ORSTOM Center in Cayenne.

EXTRACTION AND ISOLATION.—Dried leaves (425 g) were exhaustively defatted with petroleum ether and the extract (6.13 g) was conserved for testing. Defatted leaves were extracted with CH_2Cl_2 (5 liters for 60 h). The extract was concentrated *in vacuo* to give a residue (8.87 g). The leaves were then extracted with EtOAc (5 liters). The EtOAc extract was concentrated *in vacuo* to give a residue (4.51 g). These extracts, after examination in tlc, were combined and subjected to prep. hplc [CH_2Cl_2-EtOAc (85:15)] to give seven fractions which were mainly a mixture of **1** and **2** (611 mg). The purest fractions were further separated by successive prep. tlc (Si gel) and recrystallization in MeOH to yield 19.4 mg of **1** and 8.7 mg of **2**.

5,7,4′-Trihydroxyflavan [**1**].—Crystallization of **1** gave colorless crystals: mp 208–211°; uv λ max (EtOH) (log ϵ) 214 (3.74), 274 (2.58) nm; bathochromic shift of 20 nm in basic medium; ir ν max ($CHCl_3$) 3390 (OH), 1615, 1605, 825–810 (aromatic bands) cm^{-1}; eims *m/z* [M]$^+$ 258 (88) 139 (100), 133 (38), 121 (38), 120 (100), 107 (35); ^{1}H nmr ($CDCl_3$) δ 1.83 (1H, m, H-3), 2.00 (1H, m, H-3), 2.52 (2H, m, H-4), 4.18 (1H, dd, J=12.5 and 2.5 Hz, H-2), 5.67 (1H, d, J=2.5 Hz, H-6), 5.81 (1H, d, J=2.5 Hz, H-8), 6.68 (2H, d, J=9 Hz, H-3′ and H-5′), 7.12 (2H, d, J=9 Hz, H-2′ and H-6′); ^{13}C nmr (CD_3OD) δ 158.0, 157.9, 157.4, 157.3 (C-9, C-7, C-5, C-4′), 134.3 (C-10), 128.5 (C-3′, C-5′), 116.0 (C-2′, C-6′), 102.5 (C-1′), 96.0 (C-8), 95.9 (C-6), 78.8 (C-2), 30.9 (C-4), 20.5 (C-3).

Synthesis of 5,7,4′-trihydroxyflavan [**1**] was performed from narigeninin 65% yield according to the procedure described in ref. (5).

5,7-Dihydroxy-4′-methoxyflavan [**2**].—Crystallization of **2** gave colorless crystals: mp 193–196°; uv λ max (EtOH) (log ϵ) 224 (3.94), 274 (2.69) nm; ir ν max ($CHCl_3$) 3380 (HO), 1630, 1615, 805 (aromatic bands) cm^{-1}; eims *m/z* [M]$^+$ 272 (33), 147 (17), 134 (89), 121 (26), 105 (58), 91 (100); ^{1}H nmr ($CDCl_3$) δ 1.96 (1H, m, H-3), 2.10 (1H, m, H-3), 2.62 (2H, m, H-4), 3.75 (3H, s, OCH_3), 4.87 (1H, dd, J=12.5 and 2.5 Hz, H-2), 5.90 (1H, d, J=2.5 Hz, H-6), 5.95 (1H, d, J=2.5 Hz, H-8), 6.83 (2H, d, J=9 Hz, H-3′ and H-5′), 7.26 (2H, d, J=9 Hz, H-2′ and H-6′); ^{13}C nmr (CD_3OD) δ 160.6, 157.9, 157.4, 157.3 (C-4′, C-9′, C-5, C-7), 135.4 (C-10), 128.4 (C-3′ and C-5′), 114.7 (C-2′ and C-6′) 102.5 (C-1′), 96.0 (C-8), 95.9 (C-6), 78.6 (C-2), 55.7 (OCH_3), 30.8 (C-4), 20.4 (C-3).

LEISHMANICIDAL TESTS IN VITRO.—Amastigotes of the H142 Guianese strain of *L. amazonensis* were obtained from foot lesions of a sacrificed BALB/c mouse as previously described (1). An average of 10^9 amastigotes was obtained from the two foot lesions. The suspension was diluted to 15×10^6 amastigotes per ml. Peritoneal mouse macrophages were prepared according to ref. (1). One million non-inflammatory macrophages were collected from each BALB/c mouse. The cells adhered at 37° after two h under 5% CO_2 in a CO_2 incubator. The plates were rinsed two to three times with 0.5 ml of RPMI+buffer MOPS (Sigma, USA) without FCS to eliminate non-adhering cells. The supernatant on the plates was replaced by fresh medium (RPMI+glutamine+FCS+antibiotics), 0.5 ml of medium per well. For the infection of macrophages by amastigotes (1), the medium in the wells containing the macrophages was replaced by the suspension of amastigotes. Infection took place overnight with a 5:1 ratio amastigotes:macrophage. A solution of the compounds to be tested was added to the cultures maintained at 37° in 5% CO_2 for 24 h. The medium was then replaced and the cells incubated for another 24 h before fixation. The plates were fixed with MeOH and stained with 10% Giemsa's stain (Specia, France). They were

set up with Eukitt Resin (CML, France) of inclusion. The reading of macrophages with and without parasites was performed with a 40 times magnification. For each well, the percentage of parasitized cells was calculated in relation to the total number of macrophages on the plate. For each triplicate assay, the index of survival of the amastigotes was calculated relative to the control.

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