February 2003 Notes *Chem. Pharm. Bull.* **51**(2) 207—208 (2003) 207

Diterpenoids from Amazonian Crude Drug of Fabaceae

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Received September 21, 2002; accepted November 21, 2002

A new cassane diterpene was isolated from Amazonian crude drug, "Acapu," the wood of *Vouacapoua americana*, together with two known cassane furanoditerpenes, (+)-vouacapenic acid and (+)-methyl vouacapenate. The structure was characterized as cassa-13(14),15-dien-19-oic acid on the basis of spectroscopic evidence.

Key words *Vouacapoua americana*; Fabaceae; cassa-13(14),15-dien-19-oic acid; diterpenoids; vouacapenic acid; Acapu

Vouacapoua americana Aublet belonging to the family Fabaceae is the tall tree growing in Amazonian area. The wood of this plant "Acapu" is used as an anti-ulcer agent. As the constituents of this plant, cassane furanoditerpenoids, (+)-vouacapenic acid and (+)-methyl vouacapenate had been isolated by King *et al.*[1] We have isolated a new cassane diterpene and elucidated its structure.

Results and Discussion

The methylenechloride extract of Acapu yielded compounds **1**—**3** after repeated chromatographic separation procedures.

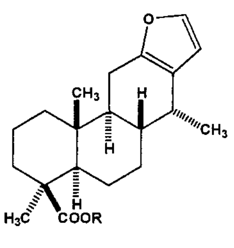
Compounds **1** and **2** gave a red color with Ehrlich reagent suggesting the presence of the furan ring in the molecular and were identified as (+)-vouacapenic acid and (+)-methyl vouacapenate, respectively, by comparison with the authentic spectral data in literature.[2—4] These structures were further confirmed by transformation to vouacapenol by methylation with diazomethane followed by reduction with $LiAlH_4$ of **1**.[1,4]

Compound **3**, colorless needles, mp 175—177 °C, $[\alpha]^{23}_{D}$ −95.5° (EtOH), gave a fluorescence quenching spot under UV_{254} light and was assigned the molecular formula $C_{20}H_{30}O_2$ by high resolution-electron impact (HR-EI)-MS (*m/z* 302.2244 $[M]^+$). The UV spectrum of **3** showed the absorption maximum at 244 nm. The IR spectrum showed the absorption band at 1725 cm^{-1}, suggesting the presence of a carboxylic acid.

The NMR spectra of **3** indicated the presence of two *tert*-methyl groups [^1H: δ 0.76 (3H, s), 1.23 (3H, s), ^{13}C: δ 12.5, 28.8], one olefin methyl group [^1H: δ 1.75 (3H, s), ^{13}C: δ 15.8, 128.5 and 136.8], seven methylene groups [^1H: δ 1.85 (1H, m) and 0.96 (1H, dt, J=4.4, 13.3 Hz), 1.90 (1H, m) and 1.50 (1H, br d, J=14.3 Hz), 2.17 (1H, br d, J=12.9 Hz) and 1.04 (1H, dt, J=4.1, 12.9 Hz), 1.90 (2H, m), 2.23 (1H, dq, J=12.6, 3.6 Hz) and 0.85 (1H, dq, J=4.4, 12.6 Hz), 1.85 (1H, m) and 1.06 (1H, dt, J=4.0, 13.3 Hz), 2.33 (1H, br d, J=17.0 Hz) and 2.00 (1H, m), ^{13}C: δ 38.8, 19.4, 37.9, 23.9, 31.9, 21.5 and 26.6] and three methine groups [^1H: δ 1.13 (1H, dd, J=12.4, 3.6 Hz), 2.00 (1H, m) and 0.88 (1H, m), ^{13}C: δ 56.1, 41.5 and 53.3]. In addition to these signals, a vinyl group [^1H: δ 6.81 (1H, dd, J=17.3, 11.0 Hz), 4.96 (1H, d, J=11.0 Hz) and 5.11 (1H, d, J=17.3 Hz), ^{13}C: δ 135.5 and 110.8], two quaternary alkane carbons (^{13}C: δ 43.8 and 37.3) and one carbonyl carbon (^{13}C: δ 184.5) were observed.

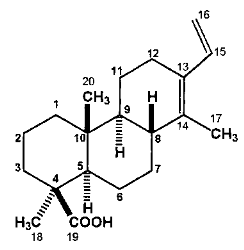
These spectral data closely resembled to those of **1** except for the presence of a 3,4-disubstituted 1,3-pentadienoic moiety instead of the furan ring. The complete assignments of all proton and carbon resonance were based on the results of ^1H–^1H shift correlation spectroscopy (^1H–^1H-COSY), ^1H-detected heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond coherence (HMBC) spectral data (Table 1).

In the HMBC spectrum of **3** (Fig. 1), the long-range correlation were observed between H-12/C-13 and 14; H-15/C-12—14 and 16; H-16*E*/C-13; H-16*Z*/C-13—15; H-17/C-8 and 12—16. From the above data, the planer structure of **3** was determined as shown in Fig. 1. The stereochemistry of the ring A—C junction and the carboxyl group of C-19 was drawn from the results of the nuclear Overhauser effect correlation spectroscopy (NOESY) experiment of **3** (Fig. 2). From above results, compounds **3** was determined as cassa-13(14),15-dien-19-oic acid.



1: R=H

2: R=CH3



3

Experimental

General mps: uncorr. The ^1H- and ^{13}C-NMR spectra were recorded on a Varian UNITY INOVA-500 spectrometer, operating at 500 MHz for proton and 125 MHz for carbon, with tetramethylsilane (TMS) as an internal standard. HR-EI-MS spectra were obtained from a Hitachi M-4100H (70 eV) mass spectrometer. UV and IR spectra were recorded on a Shimadzu UV-2100 and Perkin Elmer FT-IR 1720 spectrophotometer, respectively. Optical rotations were recorded on a JASCO J820 digital polarimeter. Column chromatography (CC) was carried out on silica gel 60 (70—230 mesh, Merck). TLC was performed on precoated silica gel 60 GF_{254} (0.25 mm, Merck) and silica gel 60 PF_{254} (1 mm, Merck).

Isolation of Diterpenes 1—3 The crushed barks of "Acapu" were obtained from the Iquitos market, Peru, through Shinwa Bussan Co., Ltd., Osaka, Japan. A voucher specimen has been deposited in the Herbarium of this University. The barks (1.8 kg) were extracted with CH_2Cl_2 and concentrated to dryness *in vacuo* to afford a brownish extract (35.3 g). The extract was dissolved in MeOH. The insoluble material was filtered and gave a mixture (1.9 g) of **1** and **2**. On the other hand, the filtrate was concentrated to dryness *in vacuo*, and chromatographed on silica gel with CH_2Cl_2 followed by preparative TLC with two solvent system, CH_2Cl_2 and hexane–EtOAc, to give **1** (0.6 g), **2** (7.1 g) and **3** (0.2 g).

Cassa-13(14),15-dien-19-oic Acid (**3**): Colorless crystals (from MeOH),

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Table 1. NMR Spectral Data of Compound **3** and **1** ($CDCl_3$)

Position	**3**				**1**	
	δ_H (ppm)[a)]	δ_C (ppm)[b)]	Multiple bond correlation	NOESY	δ_H (ppm)[a)]	δ_C (ppm)[b)]
1α	0.96 (dt, J=4.4, 13.3 Hz)	38.8 t	2, 9, 10, 20	1β, 5, 9	1.06 (dt, J=3.4, 13.7 Hz)	40.2 t
1β	1.85 (m)		3, 5, 9	1α	1.75 (m)	
2α	1.50 (br d, J=14.3 Hz)	19.4 t		2β, 3α, 3β	1.49 (br d, J=13.7 Hz)	19.8 t
2β	1.90 (m)			2α, 20	1.86 (tq, J=3,4, 13.7 Hz)	
3α	1.04 (dt, J=4.1, 12,9 Hz)	37.9 t	2, 4, 18, 19	2α, 3β, 18	1.05 (dt, J=3.4, 13.7 Hz)	38.1 t
3β	2.17 (br d, J=12.9 Hz)		1, 4, 5	2α, 3α, 18	2.19 (br d, J=13.7 Hz)	
4		43.8 s				44.4 s
5	1.13 (dd, J=12.4, 3.6 Hz)	56.1 d	3, 4, 6, 7, 10, 18, 19, 20	1α, 9, 18	1.16 (dd, J=12.4, 3.9 Hz)	56.8 d
6α	1.90 (m)	23.9 t	5, 8	6β, 7β	1.95 (dq, J=14.0, 3.9 Hz)	23.5 t
6β	1.90 (m)		5, 8	6α, 20	1.75 (m)	
7α	0.85 (dq, J=4.4, 12.6 Hz)	31.9 t	6	7β, 9	1.32 (dq, J=3.9, 13.0 Hz)	32.3 t
7β	2.23 (dq, J=12.6, 3.6 Hz)			6α, 7α, 8, 17	1.75 (m)	
8	2.00 (m)	41.5 d	13, 14	7β, 11β, 17, 20	1.75 (m)	36.2 t
9	0.88 (m)	53.3 d	1, 5, 7, 8, 10, 11, 12, 14, 20	1α, 5, 7α, 12α	1.46 (dt, J=6.6, 10.3 Hz)	45.5 d
10		37.3 s				38.5 s
11α	1.85 (m)	21.5 t	8, 9, 12, 13	11β, 12α, 12β	2.59 (dd, J=16.7, 6.6 Hz)	22.9 t
11β	1.06 (dt, J=4.0, 13.3 Hz)		8, 9, 12	8, 11α, 12β, 20	2.36 (dd, J=16.7, 10.3 Hz)	
12α	2.00 (m)	26.6 t	13, 14	9, 11α, 12β, 16Z		150.1 s
12β	2.33 (br d, J=17.0 Hz)		13, 14	11α, 11β, 12α, 16Z		
13		128.5 s				122.7 s
14		136.8 s			2.62 (dq, J=6.6, 7.1 Hz)	32.0 d
15	6.81 (dd, J=17.3, 11.0 Hz)	135.5 d	12, 13, 14, 16	17	6.16 (d, J=1.8 Hz)	110.0 d
16E	4.96 (d, J=11.0 Hz)	110.8 t	13	16Z, 17	7.21 (d, J=1.8 Hz)	140.8 d
16Z	5.11 (d, J=17.3 Hz)		13, 14, 15	12α, 12β, 16E, 17		
17	1.75 (s)	15.8 q	8, 12, 13, 14, 15, 16	7β, 8, 15, 16E, 16Z	0.97 (d, J=7.1 Hz)	18.0 q
18	1.23 (s)	28.8 q	2, 3, 4, 5, 19	3α, 3β, 5	1.27 (s)	29.6 q
19		184.5 s				185.0 s
20	0.76 (s)	12.5 q	1, 5, 9, 10	2β, 6β, 8, 11β	0.82 (s)	13.9 q

a) Values were recorded at 500 MHz; assignments from ^1H–^1H COSY, HMQC, HMBC and NOESY data. *b*) Values were recorded at 125 MHz; assignments from HMQC and HMBC experiments.

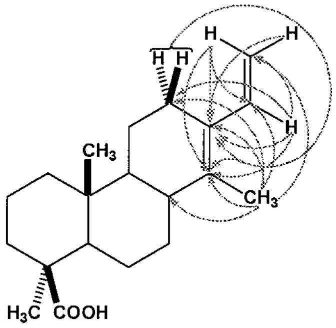

Fig. 1. The Significant HMBC Correlation of Compound **3**

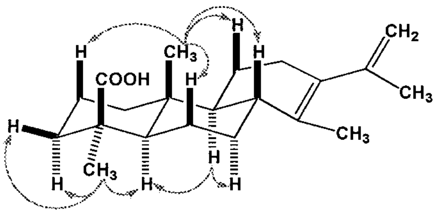

Fig. 2. The Significant NOESY Correlation of Compound **3**

mp 175—177 °C. UV λ_{max} (EtOH) nm (log ε): 244 (4.51). IR (KBr) cm^{-1}: 3370, 2948, 2848, 1725 and 1692. HR-EI-MS: *m/z* 302.2244 $[M]^+$ (Calcd for $C_{20}H_{30}O_2$: 302.2246). $[\alpha]_D^{23}$ −95.5° (c=1.05, EtOH). ^{1}H- and ^{13}C-NMR data are shown in Table 1.

References

1) King F. E., Godson D. H., King T. J., *J. Chem. Soc.*, **1955**, 1117—1125 (1955).
2) Spencer T. A., Smith R. A. J., Storm D. L., Villarica R. M., *J. Am. Chem. Soc.*, **93**, 4856—4864 (1971).
3) Bernasconi S., Gariboldi P., Jommi G., Sisti M., Tavecchia P., *J. Org. Chem.*, **46**, 3719—3721 (1981).
4) Godoy R. L. de O., Lima P. D. de D. B., Pinto A. C., De Aquino Neto F. R., *Phytochemistry*, **28**, 642—644 (1989).