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ANTITUMOR PRINCIPLES FROM CASEARIA SYLVESTRIS SW. (FLACOURTIACEAE), STRUCTURE ELUCIDATION OF NEW CLERODANE DITERPENES BY 2-D NMR SPECTROSCOPY

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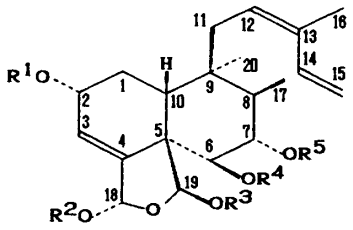
From the leaves of Casearia sylvestris Sw., a Paraguayan and Brazilian medicinal plant, six new clerodane diterpenes have been isolated and their structures have been determined by 2-D NMR spectroscopy including INADEQUATE and ^{1}H-^{13}C long-range COSY. Two of them showed strong antitumor activity against Sarcoma 180 ascites in mice.

KEYWORDS - Casearia sylvestris; Flacourtiaceae; antitumor principle; clerodane diterpene; INADEQUATE; ^{1}H-^{13}C long-range COSY

Preliminary antitumor screening tests of crude drugs and collected plants[1] have been carried out by means of the total packed cell volume method using Sarcoma 180 ascites in mice.[2] Among many species of South American medicinal plants screened in antitumor tests, the ethanolic extract prepared from the leaves of Casearia sylvestris Sw. (Paraguayan name "Burro-Kaa")[3] was particularly active (100 mg/kg/day; GR 13.2%, ++). The activity was concentrated in n-hexane extract (100 mg/kg/day; 1.7%, +++). The extract was chromatographed on a silica gel column and an octadecylsilyl column to give the new clerodane diterpenes (I - VI). The antitumor activity of compounds I-VI against Sarcoma 180 ascites in mice and their cytotoxicity against V-79 cells in vitro are summarized in Chart 1.



compound	R^1	R^2	R^3	R^4	R^5	S 180 A+	V-79*
I	CH_3	$CH_3C{=}O$	$CH_3C{=}O$	H	$CH_3(CH_2)_2C{=}O$	3.97%	1.0×10^{-3}
II	CH_3	$CH_3C{=}O$	$CH_3C{=}O$	$CH_3C{=}O$	$CH_3(CH_2)_2C{=}O$	76.85%	8.5×10^{-3}
III	H	$CH_3C{=}O$	$CH_3C{=}O$	$CH_3C{=}O$	$CH_3(CH_2)_8C{=}O$	1.89%	7.7×10^{-4}
IV	H	$CH_3(CH_2)_2C{=}O$	$CH_3C{=}O$	H	$CH_3(CH_2)_2C{=}O$	------	1.8×10^{-3}
V	H	H	$CH_3C{=}O$	CH_3CH_2	$CH_3(CH_2)_8C{=}O$	------	4.7×10^{-3}
VI	H	H	$CH_3C{=}O$	CH_3CH_2	$CH_3(CH_2)_2C{=}O$	81.26%	2.9×10^{-2}

Chart 1

+: Sarcoma 180 ascites in mice; dose, 15 mg/Kg/day; PCV, packed cell volume
GR(growth ratio)=PCV(test groups)/PCV(control groups)x100
*: V-79 (chinese hamster's lung cells) in vitro; LD_{50} mmol/l

To facilitate discussion, the structure of compound I is described first. Compound I (15 mg/Kg/day; GR 3.9%, +++), $C_{29}H_{42}O_9$, colorless plates, mp 82.0-83.0°C, $[\alpha]_D$ +40.1° in EtOH, showed IR absorption bands at 3600, 3400, 2980, 1760, 1640, 1600, 1230, 1080, 1025, and 1000 cm^{-1} in CCl_4 and UV absorption at 210 nm (ε 5500) and 235 nm (ε 9000) in EtOH. The EI-MS showed fragment ion peaks at 474 (M^+-AcOH), 414 (474-AcOH), and 326 (414-butylic acid). The ^1H- and ^{13}C-NMR and ^1H-^1H COSY spectra of I in $CDCl_3$ showed the presence of the partial

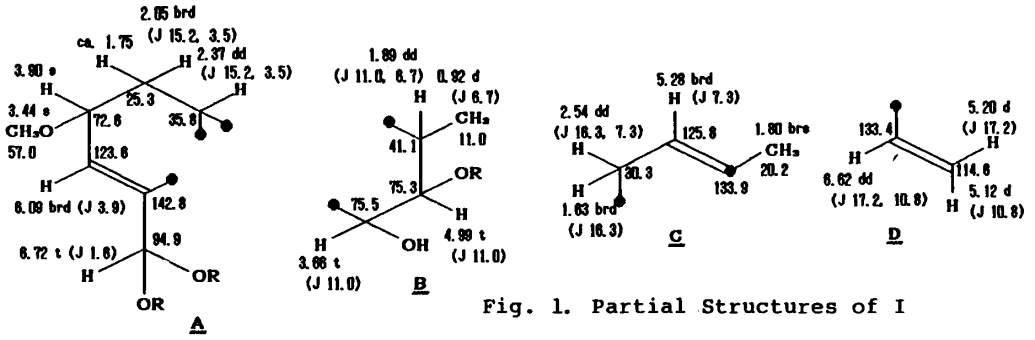


Fig. 1. Partial Structures of I

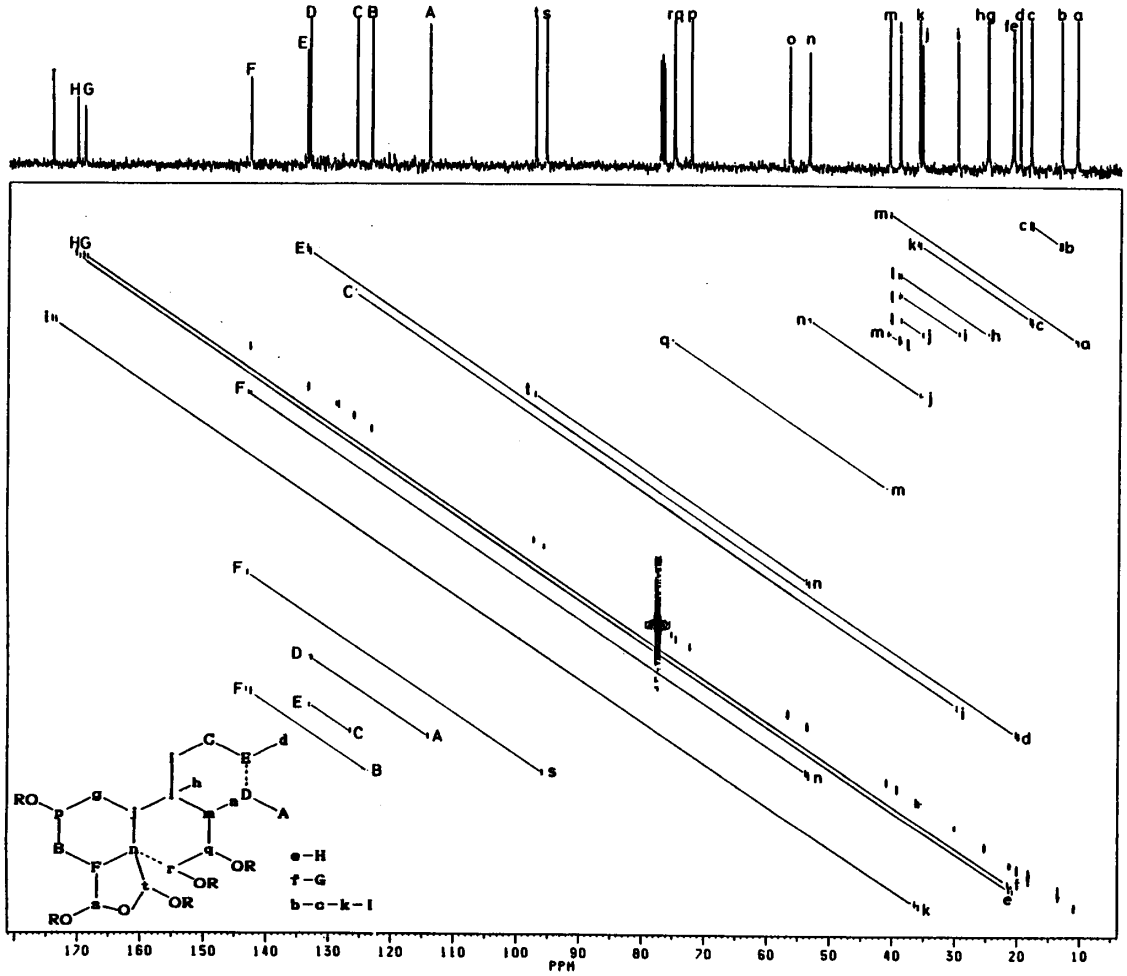


Fig. 2. INADEQUATE Spectrum of I in $CDCl_3$

The spectrum was measured on a Bruker AM-400, using 400 mg of sample (30°C, 80h run, J_{CC}=62.5 Hz). The ^{13}C spectrum runs along the lower axis; sp^2 carbons are marked with A-I and sp^3 carbons with a-t in the order of increasing values.

structures **A**, **B**, **C**, and **D** shown in Fig. 1, in addition to two acetyls, one butylate, two quarternary carbons (δC 39.4 and 53.8), one tertiary methyl (δC 25.5, δH 0.86), and one acetal carbon (δC 97.5, δH 6.55). Each carbon signal was assigned based on the ^{1}H-^{13}C COSY spectral data.

At this stage, the 2-D INADEQUATE spectrum of I in $CDCl_3$ was measured to determine the sequence of carbon atoms in the molecule. The results are shown in Fig. 2, where the correlated cross peaks shown by solid lines appear, except those between the carbons **n** and **r**, **q** and **r**, and **D** and **E**. However the sequence of carbon atoms between **q** and **r** was determined by the ^{1}H-^{1}H COSY spectrum.

We applied the ^{1}H-^{13}C long-range COSY method to I in order to determine the sequences of those carbons between **n** and **r**, between **D** and **E** and positions of the ester functions. The ^{1}H-signals at δ3.66 (**r**-H) and 1.80 (**d**-H) showed long-range correlations with the ^{13}C-signals at δ97.5 (**t**) and 133.4 (**D**), respectively. Also the ^{1}H-signals at δ6.72 (**s**-H) and 6.57 (**t**-H) showed correlation with the

TABLE I. ^{13}C-NMR Data for I - VI (100 MHz in $CDCl_3$)

No.	I	II	III	IV	V	VI
1(t)	25.3	25.4	29.5	29.5	29.5	29.5
2(d)	72.6	72.5	63.6	63.8	64.0	63.9
3(d)	123.6	125.5	127.4	125.5	125.3	125.3
4(s)	142.8	141.3	141.2	142.8	143.9	143.7
5(s)	53.8	52.9	52.8	53.8	53.7	53.6
6(d)	75.3	74.0	74.0	75.3	75.3	75.3
7(d)	75.5	72.7	72.7	75.4	75.8	75.6
8(d)	41.1	41.0	41.1	41.2	41.2	41.1
9(s)	39.4	39.3	39.3	39.5	39.5	39.4
10(d)	35.8	36.7	36.1	35.4	35.2	35.2
11(t)	30.3	30.2	30.2	30.3	30.4	30.3
12(d)	125.8	125.8	125.7	125.7	125.9	125.9
13(s)	133.9	133.9	134.1	134.0	133.9	133.9
14(d)	133.4	133.3	133.3	133.4	133.4	133.4
15(t)	114.6	114.6	114.8	114.7	114.6	114.5
16(q)	20.2	20.3	20.3	20.3	20.3	20.3
17(q)	11.1	11.0	11.0	11.1	11.1	11.1
18(d)	94.9	94.9	94.9	95.6	103.7	103.6
19(d)	97.5	97.7	97.7	97.6	97.3	97.2
20(q)	25.5	25.4	25.5	25.4	25.5	25.4
OMe	57.0(q)	57.1(q)				
OEt					15.5(q)	15.4(q)
					64.9(t)	64.9(t)
Ac	21.2(q)	21.0(q)	21.1(q)	21.3(q)	21.4(q)	21.3(q)
	170.2(s)	169.1(s)	169.0(s)	168.9(s)	169.4(s)	169.4(s)
	21.3(q)	21.2(q)	21.3(q)			
	169.0(s)	170.1(s)	170.0(s)			
		21.4(q)	21.4(q)			
		170.1(s)	170.2(s)			
ester	13.7(q)	13.8(q)	14.1(q)	13.6(q)	14.1(q)	13.7(q)
	18.6(t)	18.4(t)	22.7(t)	18.3(t)	22.7(t)	18.6(t)
	36.3(t)	36.1(t)	31.8(t)	36.4(t)	31.9(t)	36.3(t)
	174.3(s)	172.5(s)	29.4(t)	172.7(s)	29.3(t)	174.2(s)
			29.5(t)		29.4(t)	
			29.2(t)	13.7(q)	29.2(t)	
			29.2(t)	18.6(t)	29.7(t)	
			25.0(t)	26.4(t)	25.1(t)	
			34.3(t)	174.4(s)	34.5(t)	
			172.8(s)		174.4(s)	

The multiplicities of carbon signals were determined by DEPT method.

^{13}C-signals at δ170.2 (**H**) and 169.0 (**G**), respectively. Thus, the sequences of carbons and the positions of ester functions were eluciated by these 2-D NMR spectroscopy.

The relative stereochemistry of I was established on the basis of the coupling constants of each proton and the NOESY spectrum. Since the coupling constants between **r**-H and **q**-H, **q**-H and **m**-H were 11.0 Hz (**r**-H was also coupled with a hydroxyl proton, as was confirmed by ^{1}H-NMR in $CDCl_3$ with a small amount of D_2O), it is apparent that **r**-H, **q**-H, and **m**-H were alternately axial. The coupling constants of **j**-H were 15.2 Hz and 3.5 Hz indicating that **j**-H was axial. The NOE correlations among **i**-H, **t**-H, and **q**-H and between **j**-H and **C**-H indicated that **i**-H was axial and the A/B ring junction was cis and **t**-H was α. The NOE was observed between **C**-H and **d**-H and it indicated that the double bond was Z. The homoallyl coupling observed between **p**-H and **s**-H was 1.6 Hz, so **p**-H was equatrial and **s**-H was β. These findings led us to conclude that the structure of compound I was proved to be as illustrated in Chart 1.

The structures of II-VI were determined by procedures similar to those used to elucidate compound I, as shown in Chart 1. Their ester and ether function positions were estimated by ^{1}H-NMR chemical shifts of acetyl methyl functions and ^{13}C-NMR chemical shifts of corresponding signals of **p**, **s**, **t**, **r**, and **q**. The ^{13}C-NMR assignments of I-VI are shown in Table I.

The absolute configuration of III was determined by the CD spectrum of VII, which was derived from III by i)p-bromobenzoyl chloride, ii)ozone, and iii)$NaBH_4$, based on the exciton chirality method of allylic alcohol benzoate.[4] The CD spectrum of VII showed the positive Cotton curve (max 236.0 nm; Δε=12.9 in EtOH), so the absolute configuration of the C2 position of III was R, as shown in Fig. 3. Those of the other compounds were probably R, since the CD spectra of I-VI showed negative Cotton curves (max 235 nm).

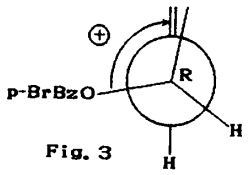
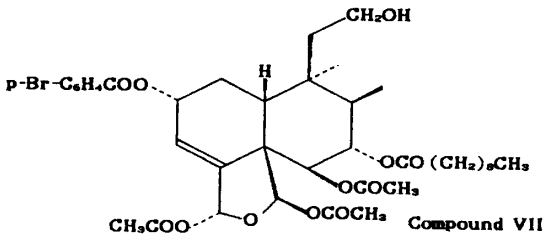


Fig. 3



Compound VII

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REFERENCES

1) H. Itokawa, K. Watanabe, and S. Mihashi, Syoyakugaku Zasshi, **33**, 95 (1979); H. Itokawa, K. Watanabe, K. Mihara, and K. Takeya, Shoyakugaku Zasshi, **36**, 145 (1982).

2) A. Hoshi and K. Kuretani, Farmacia, **9**, 464 (1973).

3) G. A. de A. B. e Silva and L. Bauer, Revista Brasileria de Farmacia, **51**(6), 327 (1970); O. Scavone, R. Grecchi, S. Panizza, and R. A. P. de S. e Silva, An. Farm. Quim. S. Paulo, **19**(1), 73 (1979).

4) N. Harada J. Iwabuchi, Y. Yokota, H. Uda, and K. Nakanishi, J. Am. Chem. Soc., **103**, 5590 (1981); N. C. Gonella, K. Nakanishi, V. S. Martin, and K. B. Sharpless, ibid., **104**, 3775 (1982).

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