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PFAFFIC ACID, A NOVEL NORTRITERPENE FROM PFAFFIA PANICULATA KUNTZE

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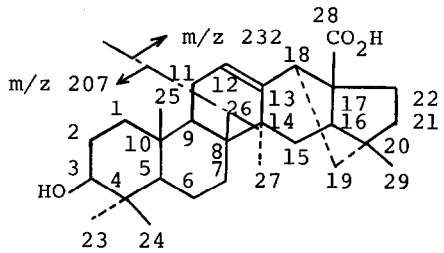
Abstract: Pfaffic acid, $C_{29}H_{44}O_3$, a new nortriterpene which exerts inhibitory effects on the growth of cultured tumor cells, was isolated from the roots of Pfaffia paniculata Kuntze and the structure established.

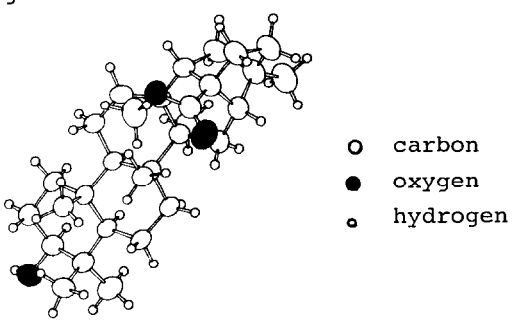
The roots of Pfaffia paniculata Kuntze (Amaranthaceae), known in Brazil as "Brazil ginseng", have been used as a tonic aphrodisiac and for antidiabetic purposes as a folk medicine[1]. We have isolated a new nortriterpene (0.006% yield from the dry roots), named pfaffic acid (I), from the methanol extract or the hydrolytic product of the saponins[2] of the roots, collected in the Goias area of Brazil[3]. Pfaffic acid (I), colorless needles (from methanol), m.p. 285-286°, $[\alpha]_D^{22}$ +109.2° (c=0.72, $CHCl_3$), IR ν_{max}^{KBr} cm^{-1}: 3350 (O-H), 1690 (C=O), shows positive Liebermann-Burchard color reaction and tetranitromethane test. The molecular formula was established as $C_{29}H_{44}O_3$ by elemental analysis and high resolution mass spectrum. The ^{1}H-NMR spectrum (d_5 pyridine) shows the presence of six tertiary methyls (0.88, 1.01, 1.10, 1.22, 1.25 and 1.47 ppm each s), one olefinic proton (5.47 ppm m) and one carbinyl proton (3.43 ppm t-like) assigned to 3α-H geminal to equatorial 3β-OH.

Further, the ^{13}C-NMR spectrum shows the presence of 29 carbons, including one carboxylic carbon (177.5 ppm), a pair of olefinic carbons (120.2 and 145.6 ppm) and one carbon bearing a hydroxyl function (78.3 ppm). The formation of following derivatives established the presence of one hydroxyl group and one carboxyl group in I : monomethylate (II), $C_{30}H_{46}O_3$, m.p. 169-170°, monoacetate (III), $C_{31}H_{46}O_4$, m.p. 302-305°, monoacetyl monomethylate (IV) and $C_{32}H_{48}O_4$, m.p. 222-224°.



pfaffic acid (I)

On the mass spectrum of I, a peak at m/z 207 agreed with that derived from the A,B rings of oleanolic acid and a peak at m/z 232, regarded as being 16 mass units less than that derived from the D,E rings of oleanolic acid, were observed. The chemical shifts of C 1-15 and C 23-28 on the ^{13}C-NMR spectrum of II were in agreement with those of methyl oleanate[4] (Table 1). Based on this evidence, I was presumed to be a hexacyclic noroleanolic acid lacking one methyl group and one hydrogen atom on the D or E ring in oleanolic acid. Finally, the structure of I was established by means of X-ray crystallographic analysis of II crystallized from methanol. Crystal data : methyl pfaffate $C_{30}H_{46}O_3 \cdot CH_3OH$, space group $P2_1$, monoclinic, a=14.317(3)Å, b=7.242(2)Å, c=13.703(3)Å, β=95.94(2)°, z=2, V=1413.2(6)Å. A total of 2362 reflections [$F_0 \geqq 3\sigma(F_0)$] were collected in the range $5° \leqq 2\theta \leqq 130°$ with a Rigaku Automated Diffractometer (AFC-5) using Cu-Kα radiation. The structure was solved by the direct method using the program MULTAN. Refinement using the least squares method gave a final R factor of 6.4%[5]. The molecular structure is shown in the figure below.



Structure of methyl pfaffate (II)

Table 1. ^{13}C-NMR chemical shifts of methyl pfaffate (II) and methyl oleanate[1*]

C, No.	II	methyl oleanate[2*]	C, No.	II	methyl oleanate[2*]
1	39.1 t	38.5	16	52.0 d[3*]	23.1
2	28.2 t	27.1	17	56.2 s	46.6
3	78.2 d	78.7	18	52.1 d[3*]	41.3
4	39.5 s	38.7	19	41.3 t	45.8
5	56.0 d	55.2	20	44.5 s	30.6
6	18.9 t	18.3	21	39.1 t	33.8
7	33.5 t	32.6	22	32.3 t	32.3
8	40.1 s	39.3	23	30.1 q	28.1
9	47.8 d	47.6	24	16.4 q	15.6
10	37.5 s	37.0	25	15.5 q	15.3
11	23.5 t	23.4	26	16.7 q	16.8
12	120.6 d	122.1	27	28.8 q	26.0
13	145.0 s	143.4	28	175.3 s	177.9
14	40.7 s	41.6	29	18.6 q	33.1
15	28.9 t	27.7	30		23.6
			$-OCH_3$	51.1 q	51.3

1*) ^{13}C FT-NMR were recorded on a JEOL FX-100 FT-NMR spectrometer. The sample was dissolved in C_5D_5N and the chemical shifts were expressed in δ-values in ppm relative to one signal (149.9 ppm) due to C_5D_5N as a standard.

2*) Reference 4.

3*) The assignments may be reversed each other.

It is very interesting that I is a novel structure based on hexacyclic nortriterpene and that it shows high inhibitory effects on the growth of cultured tumor cells, such as melanoma (B-16) and Hela (S-3) and Lewis lung carcinoma cells, at concentrations of 4-6 μg/ml, using the method devised by Takemoto et al.[6].

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NOTES

1) F.Oliveira, G.Akisue, M.k.Akisue, An. Farm. Quim. S. Paule, 20, 261 (1980).
2) Details of the saponins will be published.
3) Recently, it has been reported that 20-hydroxyecdysone was isolated from the roots of Pfaffia paniculata Kuntze collected in Sao Paulo (H.Besso, Y.Saruwatari, K.Kunihiro, T.Fuwa, R.Kasai and O.Tanaka, The 29th Annual Meeting of the Japan Society of Pharmacognosy, Sapporo, September, 1982.).
4) K.Tsuu, "Tennenbutsu Kagaku' 80A" Nankodo, Tokyo, 1980, 225.
5) Crystallographic data have been deposited with the Cambridge Crystallographic Data Center.
6) T.Takemoto, S.Arihara, S.Odashima, K.Nishikawa, N.Takagi, N.Nishimoto and S.Hayashi, Abstract Papers, the 102nd Annual Meeting of the Pharmaceutical Society of Japan, Osaka, April, 1982, p.585.

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