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ESTABLISHMENT OF THE STRUCTURE OF GYMNEMAGENIN BY X-RAY ANALYSIS AND THE STRUCTURE OF DEACYLGYMNEMIC ACID

Yoshisuke Tsuda,* Fumiyuki Kiuchi, and Hong-Min Liu
Faculty of Pharmaceutical Sciences, Kanazawa University,
13-1 Takara-machi, Kanazawa 920, Japan

Abstract: The structure of gymnemagenin, the sapogenin of antisweet principle of Gymnema syrvestre, was firmly established as 3β,16β,21β,22α,23,28-hexahydroxy-olean-12-ene by the X-ray analysis of the 3β,23;21β,22α-di-O-isopropylidene derivative. On the basis of this result, the structure of deacylgymnemic acid was elucidated as the 3-O-β-glucuronide by comparisons of the ^{13}C-NMR spectra.

Gymnemic acid, an antisweet principle of Gymnema syrvestre (Asclepiadaceae), has been considered to be a mixture of polyacylated saponins which contain glucuronic acid as a sugar moiety.[1] Although the structure of its aglycone, gymnemagenin **3**, was elucidated by Stöcklin[2] and Rao[3] mainly based on spectral evidence, it still required rigid establishment. In addition the position of glucuronic acid in deacylgymnemic acid (DAGA)[1f] as well as that of the acyl groups on the aglycone moiety also has to be clarified. We present here the rigid evidence on structure of gymnemagenin **3**[4] by X-ray analysis, and, based on this result, determine the structure of DAGA.[4]

Gymnemagenin was treated with 2,2-dimethoxypropane in acetone under catalysis of tosic acid at room temperature for 3 h to give a triacetonide, which, on treatment with chloroform, rapidly changed into a diacetonide **2**. This formed colorless prisms, mp 296-298°C, from benzene-hexane, whose single crystal was subjected to X-ray analysis.

Crystal data: $C_{36}H_{58}O_6$. M=586.85. Orthorhombic, a=22.240(2), b=22.473(3), c=6.600(2) Å. Dc=1.18 g/cm^{-3}. Z=4. Space group $P2_12_12_1$. Of the total of 3365 reflections obtained by the use of Mo-Kα radiation, 2616 reflections of the intensities above 3σ(I) level, were used in the calculation. The structure was solved by the direct method using MITHRIL[5] and refined by the full-matrix least-squares procedure with the assumption of positional anisotropic thermal parameters for all non-hydrogen atoms to afford the final R value of 0.076. An ORTEP drawing of the molecule is given in Fig. 1, which not only confirmed the proposed structure of gymnemagenin but also established the structure of the diacetonide.[6]

On the basis of established structure of gymnemagenin **3**, the ^{13}C-NMR spectra of the genin and DAGA were assigned as shown in Table 1. The large glycosylation shift[7] (+8 ppm) at C-3 in the both DAGA and its methylester (**1b**) clearly indicated the position of glucuronic acid on the aglycone. The shifts at C-23, C-4, and C-2, negative, slightly positive, and negligible, respectively, supported this assignment. The structure of gymnemagenin and that of DAGA now have the concrete basis.

References and Notes

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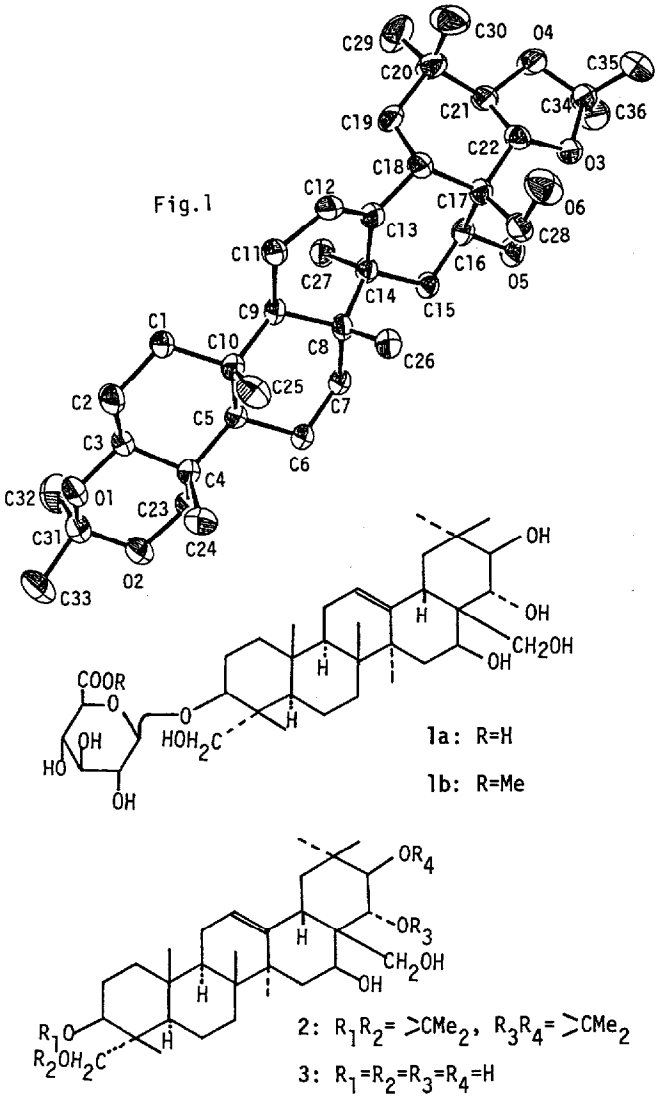
Table 1

^{13}C-NMR Data of Gymnemagenin and DAGA[8)]

C	3	1a	1b
aglycone			
1	38.6 t	39.6	40.4
2	26.3 t	26.3(+0.0)	26.0(-0.3)
3	75.3 d	83.3(+8.0)	81.4(+6.1)
4	42.0 s	42.8(+0.8)	42.4(+0.4)
5	49.2 d	48.1	47.6
6	18.4 t	18.8	18.2
7	32.4 t	33.2	32.7
8	40.1 s	41.2	40.4
9	47.0 d	48.0	47.3
10	36.9 s	37.5	36.8
11	23.8 t	24.7	24.0
12	124.2 d	125.1	123.3
13	141.2 s	142.6	142.8
14	42.6 s	43.9	43.5
15	35.0 t	36.0	36.1
16	68.6 d	69.3	68.4
17	45.7 s	47.0	46.8
18	42.1 d	43.5	42.8
19	46.1 t	47.3	46.8
20	36.2 s	37.1	36.7
21	77.1 d	76.5	77.2
22	74.2 d	74.0	74.1
23	69.8 t	64.7(-5.1)	64.6(-5.2)
24	11.8 q	13.4(+1.6)	13.5(+1.7)
25	16.0 q	16.6	16.2
26	16.8 q	17.8	17.1
27	27.3 q	27.8	27.4
28	60.3 t	59.3	59.1
29	29.6 q	30.2	30.3
30	18.3 q	18.8	18.9
sugar			
1'		105.9	106.2
2'		75.1	75.4
3'		78.2	77.9
4'		73.2	73.1
5'		77.8	77.4
6'		172.7	170.6
OMe			51.9

Fig.1



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4) Gymnemic acid obtained by Kurihara's procedure (ref. 1d) was hydrolyzed with 3% KOH-MeOH to give DAGA, mp 230-235°C, FAB-MS: m/z 683 (M^++1), which, on treatment with β-glucuronidase, gave gymnemagenin as fine prisms, mp >300°C, $[\alpha]_D^{20}$ +53.9° (c=0.75, MeOH), MS: m/z 506 (M^+). The NMR spectrum of the hexa-O-acetate, mp 298-300°C, was identical with that reported by Stöcklin (ref. 2b).
5) G.J. Gilmore, "MITHRIL: A Computer Program for the Automatic Solution of Crystal Structures for X-ray Data," University of Glasgow, Scotland, 1983.
6) Acetylation of this gave a monoacetate (δ 2.10), which on prolonged acetylation afforded a diacetate (δ 2.05 and δ 2.09), whose NMR spectrum was identical with Stöcklin's 16β,28-di-O-acetyl-3β,23;21β,22α-di-O-isopropylidene-gymnemagenin (ref. 2b).
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8) Chemical shifts in CD_3OD (**1a**), C_5D_5N (**1b**), and $CDCl_3$+CD_3OD (**3**) are given in δ ppm. Values in parentheses express glycosylation shift Δδ=δ(glycoside)-δ(genin). The multiplicity of the peaks was determined by INEPT experiment.

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