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STRUCTURE OF GLYCOCYNANCHOGENIN, A NOVEL POLYOXYPREGNANE FROM CYNANCHUM CAUDATUM MAX.

Takashi Yamagishi, Koji Hayashi, Ritsuko Kiyama, and Hiroshi Mitsuhashi
Faculty of Pharmaceutical Sciences, Hokkaido University
Sapporo, Hokkaido 060, Japan

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The structures of a number of polyoxypregnane derivatives from the rhizome of Cynanchum caudatum Max. (Asclepiadaceae) have been reported previously.1) Further survey of the polar fraction of the extract resulted in the isolation of an ester, a novel polyoxypregnane, which was named glycocynanchogenin (Ia). In the present communication, we wish to describe the structure of Ia.

Glycocynanchogenin (Ia), mp 267-277°(decomp.), $[\alpha]_D^{20}$-41.5° (c=1, MeOH), gave analytical values corresponding to $C_{28}H_{46}O_8$ in agreement with the molecular ion peak at m/e 508 in its mass spectrum. The ir and uv absorptions [ν_{max}^{Nujol} cm^{-1}: 1710, 1695, 1640, 1170; λ_{max}^{MeOH} 221 nm (ε=12000)] demonstrated the presence of a methyl-ketone and an α,ß-unsaturated ester in Ia. The nmr spectrum of Ia in CD_3OD indicated the presence of vinylic proton (δ 5.49, slightly broadened singlet), methyl-ketone (δ 2.08, singlet), vinylmethyl (δ 2.05 slightly broadened singlet), geminal dimethyl (δ 1.02, 6H, doublet, J=7 Hz), and two angular methyls (δ 1.23, 3H, singlet; 1.46 3H, singlet). The mass spectrum of Ia suggested the presence of an ikemaoyl (3,4-dimethyl-2-pentenoyl) group,2) m/e 380 (M^+-ikemaic acid), 362 (380-H_2O), and 111 ($C_7H_{11}O$). Alkaline hydrolysis of Ia with 5% methanolic KOH for 6 hr gave two neutral substances (IIa and IIb) and ikemaic acid which was identified by comparison of its ir and mass spectra with those of an authentic sample.

The two neutral substances were separated into IIa and IIb by silica gel column chromatography and showed the following physical constants: (IIa), mp 277-282°(decomp.), $C_{21}H_{34}O_7$,

ν_{max}^{Nujol} 1690 cm^{-1} (methyl-ketone), m/e 398 (M^+); (IIb), mp 257-263°(decomp.), $C_{21}H_{34}O_7$. While the Cotton effect in the region 250-350 nm in the ORD curve of IIa is negative, that of IIb is positive. These results indicate that IIa and IIb are epimeric at C-17 and IIa has a 17α-$COCH_3$ side chain.[3)] The nmr spectrum of IIa in DMSO-d_6 showed three doublets (δ 5.14, 4.32, 4.13, J=6 Hz, respectively) and these signals disappeared by addition of D_2O. This observation indicated the presence of three secondary hydroxyl groups in IIa. Considering these results, Ia is assumed to be an ester of C_{21}-steroid which has two secondary, one esterified secondary, and three tertiary hydroxyl groups, and a methyl-ketone.

The hydroxyl groups may be located at C-3ß, C-8ß, C-12ß, and C-14ß in II from the biogenetic analogy to other natural polyoxypregnanes from the same plant.[1)] These assumptions were proved by the following results. Acetylation of Ia with pyridine-acetic anhydride at room temperature for 2 days gave a monoacetate (Ib) in 47% yield, mp 155-157°, $C_{30}H_{48}O_9$, m/e 552 (M^+), and a diacetate (Ic) in 28% yield, mp 161-163°, $C_{32}H_{50}O_{10}$, m/e 594 (M^+). Under a more drastic condition (refluxing for 2 hr) Ia gave Ic, 80% yield. Methylation of Ia with Ag_2O and CH_3I in dioxane at 35° for 24 hr gave an amorphous monomethyl ether (Id) in 62% yield, m/e 394 (M^+-ikemaic acid), and a dimethyl ether (Ie) in 5% yield, mp 184-198°, m/e 408 (M^+-ikemaic acid). The nmr spectrum of Ie showed signals of 3α-proton adjacent to methoxyl group (δ 3.68, 1H, multiplet, half-band width, ca. 20 Hz)[4)] and a 12α-proton adjacent to ikemaoyl group (δ4.58, double doublet, J=5 and 10 Hz). The presence of a glycol at 8ß and 14ß in Ia was confirmed chemically by the formation of a five-membered cyclic sulfite (III). Treatment of Ic with $SOCl_2$ in pyridine at -15° afforded the cyclic sulfite (III), accompanying dehydration, mp 200-202/220-222° (double melting point), $C_{32}H_{46}O_{10}S$, m/e 622 (M^+), whose ir spectrum[5)] had no OH band but had a five-membered cyclic sulfite at 1220 cm^{-1}. In the nmr spectrum of III in $CDCl_3$, signal of vinylic proton (δ5.41, broad singlet) appeared newly. This chemical shift and splitting pattern indicated that a double bond was located at C-4 in III. According to the Dreiding model, dihedral angle between 3α proton and C-4 olefinic proton is approximately 90°, so that the observed coupling constant is ca. 0 Hz. Thus the hydroxyl group must be located at C-5 in I. As Fig. 2 shows, this was supported by the mass spectra of Ia, Id, and Ie.[6)] The configuration of C-5 hydroxyl group is assigned as α-orientation by the half-band width of the signal of 3α proton (ca. 20 Hz at δ 5.27).[4)]

The remaining problems are the stereochemistry at C-17 and location of a secondary hydroxyl group which resisted acetylation and methylation. They were established from the following evidences. The ORD curve of Ia in dioxane shows a negative Cotton effect. The similarity of the curve in the region of 250-350 nm to that of cynanchogenin (IV) indicates the α-orientation of the C-17 methyl-ketone. The signal due to 19-methyl of Ic in its nmr spectrum is shifted to a lower field than that of IV (Table). Thus the C-19 methyl group and hydroxyl group in Ic were located at 1- and 3-positions and the C-O bond is probably nearly parallel to the C-CH_3 bond.[7] From these results and the splitting pattern of the proton adjacent to the hydroxyl group (broad singlet)[8] the location of the hydroxyl group was assumed as 6β.

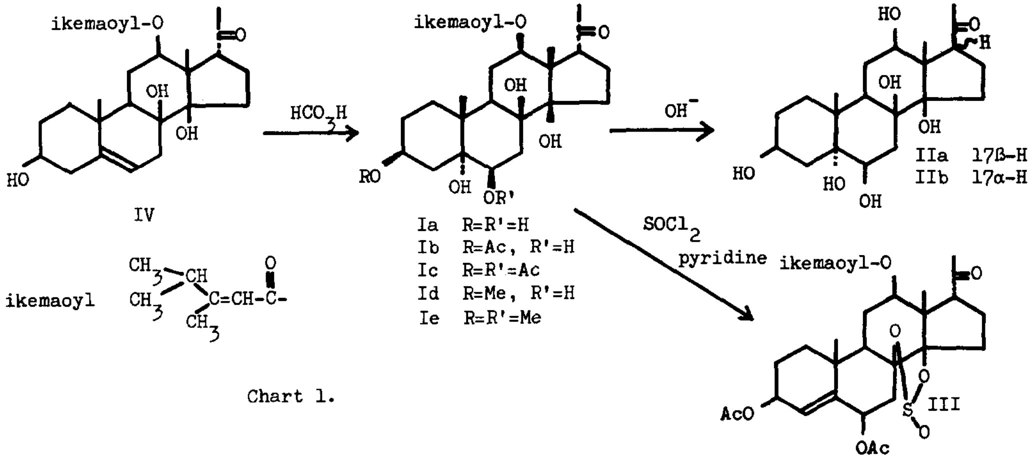

Chart 1.

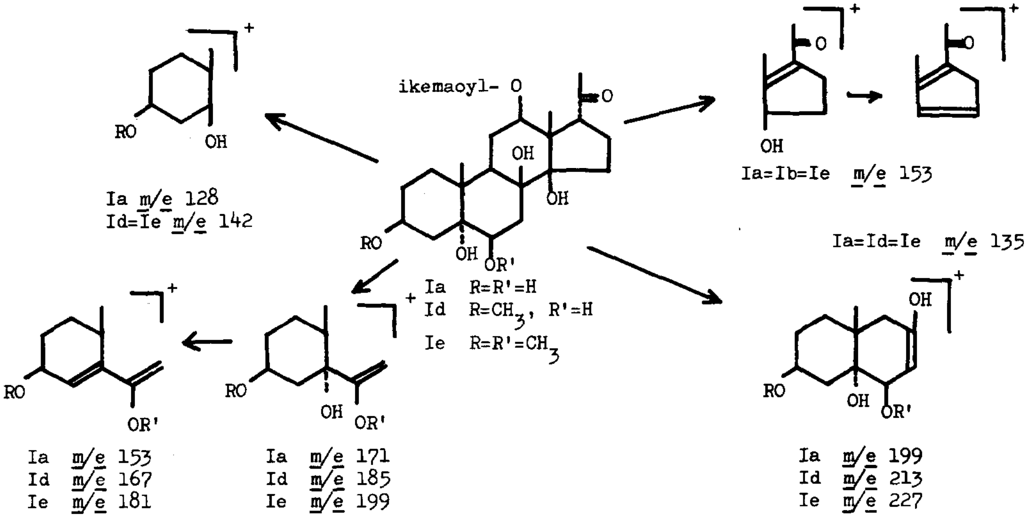

Chart 2.

Principal mass fragmentation of glycocynanchogenin

The structure of glycocynanchogenin including its stereochemistry was deduced as (Ia). This structure was confirmed by the correlation of Ia with IV. Cynanchogenin (IV) was hydroxylated according to the procedure of Fieser and Rajagopalan.[9] The crystalline product (yield, 34%) was identified by mixed melting point and by comparison of its ir, mass, and nmr spectra with those of Ia. We believe that glycocynanchogenin is the first example of a naturally occurring steroid with a glycol at 5α and 6ß.

Table. Nmr spectral data

	C-3α-H	C-6α-H	C-12α-H	C-18-CH_3	C-19-CH_3
Ib	5.13	3.70	4.57	1.50	1.30
Ic	5.14	4.94	4.62	1.49	1.25
Id	3.68	3.72	4.56	1.49	1.26
Ie	3.60	3.18	4.58	1.48	1.16
III	5.26	5.47	4.50	1.44	1.36
IV	3.54	--	4.60	1.53	1.12

Nmr spectra were taken in $CDCl_3$ and expressed in δ value using tetramethylsilane as an internal standard.

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