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THE STRUCTURES OF GLAUCOGENIN-A, GLAUCOGENIN-B, AND GLAUCOGENIN-C MONO D-THEVETOSIDE FROM CHINESE DRUG "PAI-CH'IEN" CYNANCHUM GLAUCESCENS HAND-MAZZ

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Abstract The structures of glaucogenin-A (1), glaucogenin-B (2), and glaucogenin-C mono D-thevetoside (7) were characterized by chemical and spectroscopic evidence and that of 7 determined by X-ray crystallography

Chinese crude drug "Pai-Ch'ien"[1) 芫花叶白前] ,dried root of Cynanchum glaucescens Hand-Mazz (Asclepiadaceae) has been used as an antitussive and expectorant in China This paper deals with the isolation and structural elucidation of three new compounds named glaucogenin-A (1), glaucogenin-B (2), and glaucogenin-C mono D-thevetoside (7) with novel 13,14 14,15-disecopregnane-type skeleton

The CHCl$_3$ extract of this drug showed positive Liebermann-Burchard and Keller-Kiliani reactions, suggesting the presence of steroidal glycosides containing 2-deoxy sugars The hexane-benzene (1 1) and benzene soluble portions of the extract, which are rather abundant of glycosides by the color reactions, were hydrolyzed under the milder condition (0 05 N H$_2$SO$_4$-75% MeOH, 50° , 30min) than that of usually used for glycosides of Asclepiadaceae plants.[2)] Then the hydrolysate was subjected to repeated silica gel column chromatography to give 1, 2, and 7 It was evident that the aglycone moieties of the glycosides survived under the above acidic condition because 1, 2, and 7 retained the same spectroscopic features as those of the aglycone moieties of glaucoside-A, -B, -C, -D, and -E, whose structures will be reported in the separate paper, isolated from this material

Colorless needles of 1 melted at 225-231° ,$[\alpha]_D$ +78.1° (c=1 07, MeOH), C$_{21}$H$_{28}$O$_6$ (combustion and EI-MS m/z 376 (M$^+$)), IR(CHCl$_3$) cm^{-1} 3600, 3400 (OH), 1730 (-C(=O)O-), 1710, 1655 (-C=C-O-) The ^{1}H-NMR (CDCl$_3$) spectrum showed two tertiary methyl signals at δ 0 97 and 1 54 (each 3H, s), two hydroxy-methine signals at δ 3 36 and 3 70 (each 1H, m), a methine proton signal at δ 3 46 (1H, dd, J=8, 2 Hz), signals attributable to protons carrying oxygen atoms at δ 3 88 (1H, dd, J=10, 9 Hz), 4 20 (1H, dd, J=9, 7 Hz), and 5 35 (1H, ddd, J=10, 8, 7 Hz), two olefinic proton signals at δ 5 50 (1H,d, J=4.5 Hz) and 6 27 (1H, d, J=2 Hz) being in good agreement with vincetogenin [3)], whose structure has not been determined yet, except for its specific rotation ^{13}C-NMR (Table I) and ^{1}H-NMR spectra of 1 suggested the presence of two trisubstituted double bonds and a ketal function Since 1 displayed no UV absorption corresponding to conjugated dienes or enones, its deshielded ^{1}H-NMR signal at δ 6 27 is ascribable to an enol ether function On treatment of 1 with $LiAlH_4$ in THF gave a tetrol (4), an oil, $[\alpha]_D$ -55 0°(c=1 20, MeOH), $C_{21}H_{32}O_6$, whose ^{1}H-NMR spectrum exhibited the up-field shifted signal at δ 4 52 (1H, ddd, J=10,8,6 Hz), and ^{13}C-NMR signal at δ 71.5 (t) indicating the formation of a hydroxyl methyl group by the reduction, thus suggested the existence of a lactone ring in 1 The tetrol (4) furnished a tetraacetate (5), an oil, $[\alpha]_D$ -63 0° (c=1 17, $CHCl_3$), $C_{29}H_{40}O_{10}$, by acetylation in the usual manner, and the vicinity of the acetoxyl methyl group and methine carbon was inferred from its ^{1}H-NMR signal at δ 3.92 (1H, dd, J=11 5, 7 Hz) and 4 22 (1H, dd, J=11 5, 4 Hz) Catalytic hydrogenation (H_2/PtO_2 in AcOH) of 1 afforded a tetrahydro derivative (6), mp 217-220 °, $[\alpha]_D$ +14 0°(c=0.84, $CHCl_3$), $C_{21}H_{32}O_6$

Glaucogenin-B (2), mp 269-272 5 °, $[\alpha]_D$ +135 °(c=0.23, MeOH), $C_{21}H_{28}O_7$, was denoted to bear an additional secondary hydroxyl group, compared with 1, on the bases of its ^{13}C-NMR signal at δ 67.8 (d) and yielding a triacetate (3), mp 256-258° ,$[\alpha]_D$ +82 7° (c=0 37, $CHCl_3$), $C_{27}H_{34}O_{10}$, by acetylation Proton spin decoupling experiments were carried out throughly in 3 to provide two partial structures as shown in Fig 1, being supposed to be common to 1 and 2

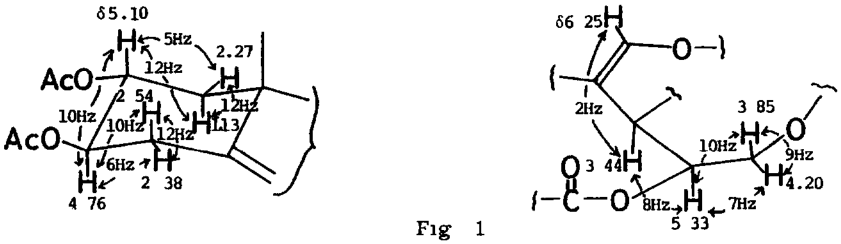
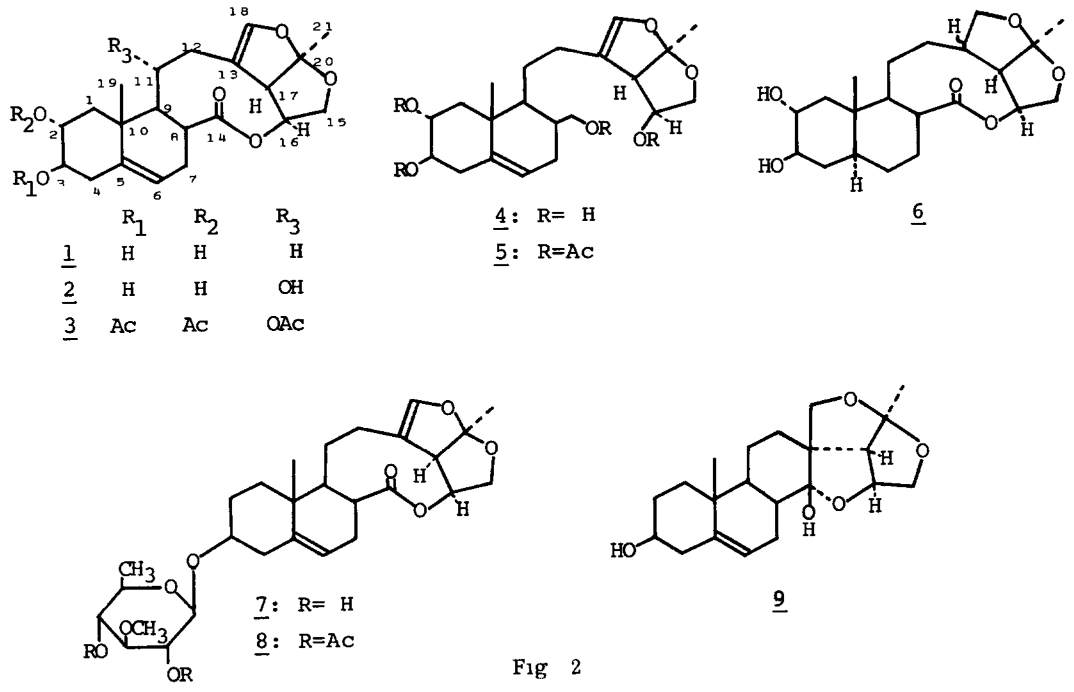


Fig 1

The thevetoside (7), mp 187-190.5° ,$[\alpha]_D$ +27 4°(c=1 03, $CHCl_3$), $C_{28}H_{40}O_9$, has similar spectroscopic features in its aglycone moiety as 1 and 2 On hydrolysis under a condition (1N H_2SO_4-50% MeOH, reflux for six hr), 7 afforded D-thevetose[4] ($[\alpha]_D$ +30° (c=0 4, H_2O)), While an attempt to obtain the aglycone remained unsuccessful. However, consideration of the ^{13}C-NMR spectrum of 7 led us to suppose that the aglycone moiety possesses the sole secondary hydroxyl group to which the sugar linked The β-linkage of the sugar was indicated by the anomeric proton signal at δ 4 35 with coupling constant 8 Hz in the ^{1}H-NMR spectrum of 7 Since there was no further improvement for the structures of 1, 2, and 7, an X-ray analysis was performed by using crystals of 8 from MeOH, the diacetate of 7, to determine the structure and relative stereochemistry unequivocally Crystal data Crystals of 8 [$C_{32}H_{44}O_{11}$] are orthorhombic, a=19 270 (6), b=23 071 (8), c=7 155 (3) Å , V=3181 Å^3 , space group $P2_12_12_1$, Z=4, D_x=1 26 g/cm^3 , Cu- Kα radiation The structure was resolved by direct method and refined to give an R value of 0 1186[5] The molecular structure is shown in Fig 4 The absolute configuration was determined as depicted in Fig 2 on the ground that the thevetose belongs to D series[4]

The close analogy of the spectroscopic features and the assumption of biogenetic similarity among 1, 2, and the aglycone moiety of 7 permitted to illustrate the structure of glaucogenin-A (1) as in Fig 2 In the ^{1}H-NMR spectrum of 3, the remaining signal due to an acetoxy- methine proton appeared at δ 5 70 (1H, dt, J=10, 2 Hz) The rather large coupling constant (10 Hz) may be based on coupling between $H_{9\alpha}$ and $H_{11\beta}$, and the second coupling between $H_{11\beta}$ and C-12-methylene Therefore, the structure of glaucogenin-B (2) was tentatively deduced as in Fig 2.

Compound 1, 2, and 7 have unprecedent skeleton namely 15,20α 18,20β-diepoxy-13,14 14,15-disecopregna-5, 13(18) dieno-14 oic acid-16 oxy-lactone The biogenisis of the nine-membered lactone ring in them may be speculated as shown in Fig 3, starting from hirundigenin (9), followed by hydroxylation at C-18, though 9 has not been detected yet in this material. It is known that the same type of lactonization as shown in Fig 3 occurs as chemical reaction in the case of ryanodine $^{6)}$ or diterpenes in Cinnamomum cassia $^{7)}$



	R1	R2	R3
1	H	H	H
2	H	H	OH
3	Ac	Ac	OAc

4: R= H
5: R=Ac

6

7: R= H
8: R=Ac

9

Fig 2

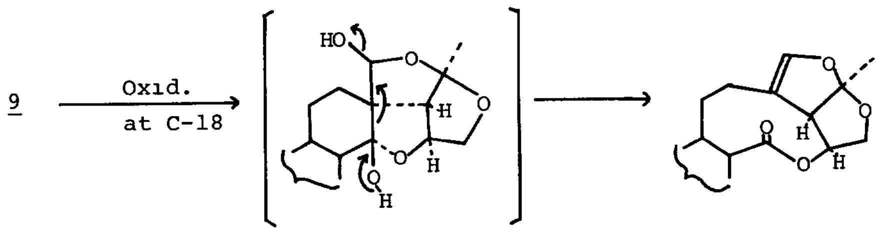


Fig 3

Table I ^{13}C-NMR Chemical Shifts for 1,2,4,6, and 7

	1	2	4	6	7
C- 1	45.5	45.3	45.9	42.9	36.6
C- 2	72.9	73.2	72.5	72.6	30.6
C- 3	76.3	76.6	76.8	76.3	78.1
C- 4	40.1	40.1	40.7	36.7	39.0
C- 5	140.9	141.6	140.8	45.6	140.7
C- 6	120.0	123.8	121.6	27.2	120.4
C- 7	30.1	23.6	28.0	28.3	30.0
C- 8	53.2	51.4	49.0	53.2	53.3
C- 9	40.4	50.3	38.2	44.5	40.7
C-10	40.4	40.1	40.4	38.7	38.7
C-11	23.9	67.8	28.4	23.1	23.9
C-12	28.2	30.2	30.6	23.5	28.4
C-13	118.5	118.6	118.7	43.5	118.4
C-14	175.4	174.9	71.5	175.3	175.4
C-15	67.8	67.9	64.9	72.4	67.7
C-16	75.5	75.8	74.2	73.4	75.5
C-17	56.2	56.4	55.4	51.6	56.2
C-18	143.8	144.0	141.3	70.8	143.8
C-19	19.2	19.0	19.8	12.9	18.6
C-20	114.3	114.5	115.3	116.7	114.3
C-21	24.8	24.8	25.2	24.9	24.8
C- 1'					102.4
C- 2'					75.0
C- 3'					88.0
C- 4'					75.9
C- 5'					72.6
C- 6'					17.9
-OMe					60.8

(in pyridine-d_5)

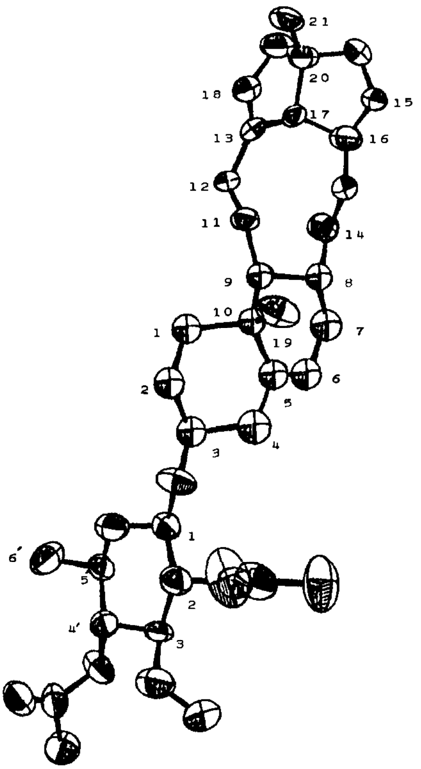


Fig. 4

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