Absolute configuration of calogenin and its 20-keto derivative

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The absolute configuration of calogenin (isolated from Periploca calophylla) and its 20-keto derivative (isolated from Hemedesmus indicus) has been established as pregn-5-ene-3β,14β,20R triol containing a C-17 hydroxy ethyl chain in α-configuration and pregn-5-ene-20-keto-3β,14β-diol containing a C-17 methyl keto chain in β-configuration, respectively.

Keywords: Asclepiadaceae, Periploca calophylla, Hemedesmus indicus, calogenin, 20-keto derivative, absolute configuration

Pregnanes and their glycosides are established as an important class of biologically active compounds. They have been isolated from various plant species, mostly belonging to the Asclepiadaceae family and have shown antitumor, anticancer, antiepilepsy, cytotoxic, antiasthmatic, antitrachices and fertility regulating activities. The condurango glycosides (CG) Ao, (CG) Bo, (CG) Co, (CG) Do, 20-iso-O-methyl condurango glycoside (CG) Co and 20-iso-O-methyl condurango glycoside (CG) Do from Marsdenia condurango were found active against Ehrlich ascites carcinoma, while two other condurango glycosides E₀₁ and E₀₂ have shown anticarcinogenic activity. During the course of chemical investigation of Asclepiadaceae plants, the absolute configuration of calogenin and its 20-keto derivative are reported.

The C₂₁ pregnane moieties contain the usual steroid moiety with a two-carbon chain at C₁₇ and commonly containing a double bond between C₅ and C₆ but in cases where this unsaturation does not exist, A/B rings fusion is usually trans. A number of 14β hydroxyl polyoxypregnane derivatives containing either a C-20 carbino or C-20 keto group, have been isolated from these plants. The polyhydroxy pregnanes have so far been found to contain hydroxyl groups at C-3, C-12, C-16 and C-17 positions in β-configuration but the C-11 hydroxyl group, if present, is of α-configuration unlike those obtained from animal sources. A characteristic feature of a C-17 keto methyl pregnanes, in the absence of any other substituent at C-17 but a C-14β hydroxyl group, is their property of undergoing isomerization α ↔ β in acid or alkaline conditions giving predominantly stabler 17α-chain. It is further reported that when C-17 has a substituent group, it is always a β-hydroxy group resulting in α-configuration of C-17 chain. It is also interesting that the absolute configuration of the hydroxyl group at C-20 varies in different compounds. To date the C-20 configuration has been established only in few of the isolated pregnanes of this class. The absolute configuration of C-17 methyl keto chain and its reduced product, C-20 carbino pregnanes in other type need to be fixed before the complete structure of a polyoxypregnane can be taken as fully established.

The configuration of C-17 methyl keto chain can be ascertained from its Optical Rotatory Dispersion (ORD) observations which is reported to exhibit a negative cotton effect when the methyl keto chain is in α-configuration and positive cotton effect when it is in β-configuration. The configuration of C-17 hydroxy ethyl chain, however, cannot be determined through its ORD study due to the absence of the needed carbonyl chromophore. For these compounds, techniques of preparing the 2-nitro benzoate of C-20 hydroxy group and their ORD studies can be adopted for fixing their C-17 chain configuration.

In the light of foregoing observation and the findings of stereochemical conversions, the authors have established the absolute configuration of calogenin 2 and its 20 methyl keto derivative 1 with a C-17 hydroxy ethyl chain in α-configuration and in β-configuration, respectively. The former was isolated from Periploca calophylla (Asclepiadaceae family) and latter from Hemedesmus indicus (Asclepiadaceae family).

Results and Discussion

The structure of compounds 1 and 2 have been elucidated earlier as pregn-5-ene-20-keto-3β,14β diol and pregn-5-ene-3β,14β,20 triol, respectively,
but the configuration of C-17 chain in 1 and 2 as well as the absolute configuration of C-20 carbinol group in 2 was still to be established.

The positive cotton effect shown by 1 led to the assignment of β-configuration for the C-17 methyl keto chain in it. Acetylation of 1 with acetic anhydride in pyridine afforded two mono-O-acetyl products (TLC), 3 (major), m.p. 171° and 4 (minor), m.p. 165°, presumably formed due to C-17 chain configurational isomerization in the presence of basic pyridine in the reaction-mixture. The major product 3 is presumed to contain a 17α chain and the product 4 evidently its 17β isomer formed as a minor component as reported in such isomerization of C-14β hydroxyl, C-17 methyl keto pregnanes. This was ascertained by the regeneration of original 17β-20-keto compound 1 on Zemplen hydrolysis of product 4.

Mitsuhashi et al. interpreted that the stereochemistry of C-20 carbinol obtained by NaBH₄ reduction of the corresponding 20-ketone is depended on the configuration of C-17 methyl keto chain. Thus a pregnane derivative with an α-methyl keto chain is expected to afford predominantly C-20 hydroxy compound of S-configuration and C-17β methyl keto pregnane will yield the NaBH₄ reduced C-20 hydroxy compound of R-configuration. The reduction of 3 with NaBH₄ evidently gave C-17 hydroxy ethyl chain also in α-configuration. The two products obtained in this reaction could, therefore, be C-20 R and S isomers. To confirm our derivation that the compounds 5 and 6 are S and R isomers, were subjected to 2-nitrobenzoylation at C-20 carbinol, giving products 9 and 10, respectively (Scheme I). The results of ORD studies agree with our derived expectation as compounds 9 and 10 showed positive and negative cotton effect respectively, leading to the conclusion that the configuration at C-20 hydroxy group in product 9 is S and 10 is R. In view of the above facts, 3 containing C-17α methyl keto chain is expected to afford on NaBH₄ reduction 5 possessing C-20 in S-configuration as a major product, whereas the minor product 6, could have the C-20R configuration. As compound 6, on alkaline hydrolysis gave calogenin 2, it could be established that the absolute configuration of 2 is pregn-5 ene, 3β, 14β, 20R triol having hydroxy ethyl chain in α-configuration.

Scheme I — (Ar=2-nitrophenyl)
Similarly, the reduction of the minor product 4 containing C-17β methyl keto chain, with NaBH₄ showed two products on TLC, 7 (major) and 8 (minor). Chromatographic separation afforded only compound 7, which was then esterified at C-20 with 2-nitrobenzoyl chloride giving product 11. The compound 8 could not be isolated being a minor constituent of minor 4. The results of ORD studies again agree to our expectation as compound 11 showed negative cotton effect leading to the conclusion that the configuration at C-20 is R. Again, according to Mitsuhashi interpretation, 4 containing C-17β methyl keto chain is expected to afford on NaBH₄ reduction, the major product 7 possessing C-20 in R configuration, whereas the minor product 8, could have the C-20 in S configuration (Scheme II).

Experimental Section

The general procedures were the same as those reported earlier.

Acetylation of 1. A solution of 1 (22 mg), [α]D²⁵ +438.24, in pyridine (1 mL) and acetic anhydride (1 mL) was heated at 80° for 4 h. Usual work-up yielded two mono-O-acetyl derivatives: 3 (16 mg, major), m.p. 171°C and 4 (5 mg, minor), m.p. 165°C.

Alkaline hydrolysis of 4 by Zemplen method. To a solution of 4 (0.5 mg) in absolute methanol (0.2 mL) was added sodium methoxide (0.04 mL) and the mixture was kept at RT. After 15 min, it was neutralised with IR 120 H⁺ resin, filtered and yielded residue 1 (TLC).

NaBH₄ reduction of 3. Compound 3 (15 mg) in methanol (1.5 mL) was reduced with sodium borohydride (12 mg). After keeping it at RT, for 2 hr, the pH was brought to 8-9 by adding 6% alcoholic acetic acid, kept for 15 h at RT. and excess of borohydride was decomposed with acetic acid. Water (13 mL) was added and methanol was removed under red. pres. The aq. concentrate was extracted with 3 x 2 mL chloroform: methanol (90:10) and organic layer washed with water (0.5 mL), dried and evaporated yielding residue containing two products (TLC). These products were separated on silica gel column affording 5, [α]D +46.7°, (9 mg, major, Rₚ 0.40) and 6, [α]D -87.5°(3.5 mg, minor, Rₚ 0.45).

2-Nitrobenzoylation of 5. To a solution of 5 (8 mg) in pyridine (0.5 mL), 5 mg of 2-nitrobenzoyl chloride was added and the mixture was stirred for 16 h at RT. The mixture was poured into ice water and extracted with ether. The ether solution was washed successively with 2N HCl, 5% NaHCO₃ and water and dried over Na₂SO₄ to give compound 9 (6.5 mg), [α]²⁹₀ +1197.90°.

2-Nitrobenzoylation of 6. To a solution of 6 (3 mg) in pyridine (0.3 mL), 3 mg of 2-nitrobenzoyl chloride was added and the mixture was stirred for 16 h at RT. The usual work-up as done in the 2-nitrobenzoylation of 5 afforded compound 10 (2 mg), [α]²⁹₀ -21242.76°.

NaBH₄ reduction of 4. Compound 4 (4 mg) in methanol (1 mL) was reduced with sodium borohydride (3 mg). The working of the reaction-mixture, as done in the sodium borohydride reduction of 3, followed by silica gel column, afforded 7, [α]D -91.3° (3 mg, major, Rₚ 0.30). The other isomer 8 could not be isolated due to the meager quantity.
2-Nitrobenzoylation of 7. To a solution of 7 (3 mg) in pyridine (0.3 mL), 3 mg of 2-nitrobenzoyl chloride was added and the mixture was stirred for 16 h at RT. The usual work-up as done in the 2-nitrobenzoylation of 5, yielded compound 11 (2 mg), $[\alpha]_{290}^{25} = -2726.9^\circ$.

Reference