[Chem. Pharm. Bull. 16(7)1407—1409(1968)]

UDC 547.92.02.04

The Configuration of 22-Hydroxycholesterol

In the course of the studies on the synthesis of ecdysone (I), two 22-hydroxy-3,6-dioxohomo-5α-cholan-25-oic acid 25→22-lactones (II and III) epimeric at asymmetric center of C-22 were prepared as a starting material for the synthesis.[1] The establishment of the configuration of the hydroxyl group at C-22 was very important at this point, because the compound in which the configuration under consideration is the same as that of ecdysone, had to be selected as a starting material. Moreover, if the establishment of the configuration in these lactones and the synthesis of ecdysone (I) from one of these lactones are succeeded, the chemical proof of the configuration of 22-hydroxyl group in ecdysone (I) will be given. Klyne and Stokes[2] reported that 22-hydroxycholesterol (IV) isolated from *Narthecium ossifragum* by Stabursvik[3] should be formulated as 5-cholestene-3β,22α_F-diol, and this conclusion was supported by Tsuda and Hayatsu.[4] As this conclusion seemed to be generally accepted, an attempt to correlate dioxolactones (II and III) with 22-hydroxycholesterol was made, which will be described in this communication.

It was reported[1] that dioxolactones, II (mp 243—246°, $[\alpha]_{578}^{20}$ +20°) and III (mp 270—277°, $[\alpha]_D^{31}$ +28°) can be transformed into the corresponding 22,25-dihydroxy-5α-cholestane-3,6-diones, V (mp 203—206°, $[\alpha]_D^{26}$ +7°) and VI (mp 184.5—186.5°, $[\alpha]_D^{26}$ −9°), respectively by three steps of reactions. These two compounds are key intermediates of transformations described in this communication.

The selective reduction of the dihydroxydione (VI) with sodium borohydride in dichloromethane-ethanol mixture[5] afforded 3β,22,25-trihydroxy-6-one (VII, 209.5—211°, $[\alpha]_D^{16}$ −20°), the structures of which was assigned by analogy with the observation on the other steroid 3,6-diones. The treatment of VII with acetic anhydride and ethyl acetate containing a small amount of perchloric acid gave an oily product (VIII). This product was found to be a mixture of 3β,22-diacetoxy-5α-cholest-24-en-6-one (main product) and 3β,22-diacetoxy-5α-cholest-25-en-6-one by thin-layer chromatography, infrared and nuclear magnetic resonance spectrum. Without the isolation of any isomer in pure state, the diacetate (VIII) was treated with ethanedithiol and boron trifluoride etherate to give an oily thioketal (IX), which on reduction with W-2 Raney nickel in boiling ethanol yielded 3β,22-diacetoxy-5α-cholestane (Xa, mp 110—113°, $[\alpha]_{578}^{16}$ −6°) by reductive desfulfuration accompanied by the hydrogenation of the double bond in the side chain. The reductive hydrolysis of Xa with lithium aluminum hydride gave 5α-cholestane-3β,22-diol (Xb, mp 175—178°, $[\alpha]_{578}^{16}$ +3°). The physical constants of this diol and its acetate (Xa and Xb) are in quite agreement with those reported by Fieser and Huang[6] (diol; mp 180—182°, $[\alpha]_D$ +8°. diacetate; mp 125—127°, $[\alpha]_D$ −10°), which are apparently different from those of the corresponding compounds derived from natural 22-hydroxycholesterol.[3] The diol (Xb) was oxidized with Jones reagent to lead 5α-cholestane-3,22-dione (XI), which was identical with an authentic sample in all respects, proving definitively the diol (Xb) to be 5α-cholestane-3β,22-diol.

The similar transformations were made on another dihydroxydione (V) and the following compounds were obtained. 3β,22,25-trihydroxy-6-one (XII, mp 225—228°, $[\alpha]_D^{16}$ −7°),

1) H. Mori, K. Shibata, K. Tsuneda, and M. Sawai, *Chem. Pharm. Bull.* (Tokyo), to be submitted.
2) W. Klyne, and W.M. Stokes, *J. Chem. Soc.*, **1954**, 1979.
3) A. Stabursvik, *Acta Chem. Scand.*, **7**, 1220 (1953).
4) K. Tsuda and R. Hayatsu, *Chem. Pharm. Bull.* (Tokyo), **6**, 580 (1958); *Idem*, *J. Am. Chem. Soc.*, **81**, 5987 (1959).
5) H. Mori, K. Shibata, K. Tsuneda, and M. Sawai, *Chem. Pharm. Bull.* (Tokyo), **16**, 563 (1968).
6) L.F. Fieser and W.Y. Huang, *J. Am. Chem. Soc.*, **75**, 5356 (1953).

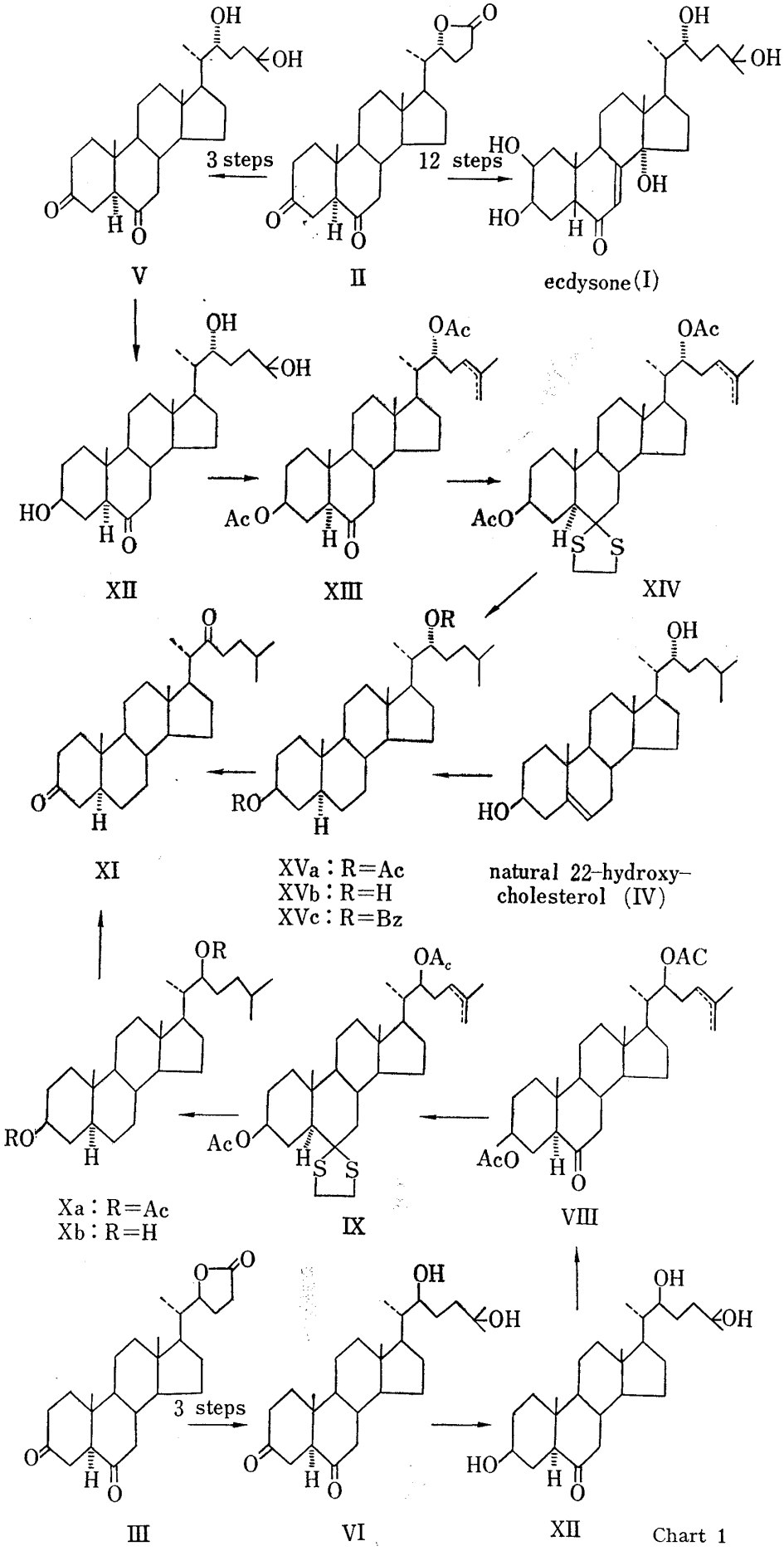


Chart 1

diacetate and thioketal (XIII and XIV, as mixtures), 3β,22–diacetoxy–5α–cholestane (XVa, oily), 5α–cholestane–3β,22–diol (XVb, mp 168—170°, $[\alpha]_{578}^{16}$ +18°), 3β,22–dibenzoyloxy–5α–cholestane (XVc, mp 205—207°, $[\alpha]_{578}^{16}$ +17°). The diol (XVb) and its dibenzoate (XVc) were found to be identical with authentic samples of the correspoinding compounds derived from natural 22–hydroxycholesterol (IV) by direct comparison.

The synthesis of ecdysone from the dioxolactone (II) and 22–isoecdysone from another dioxolactone (III) have already been accomplished.[5,7] Accordingly, it is concluded that the configuration of the 22–hydroxyl group in natural 22–hydroxycholesterol (IV) is the same as that of ecdysone (I). If the configuration assgined in ecdysone is considered to be unequivocal, natural 22–hydroxycholesterol must be formulated as 5–cholestene–3β,22β_F–diol.

Acknowledgement The authors wish to express their sincere thanks to Dr. A. Stabursvik for providing valuable samples. They are also indebted to Drs. I. Chuman, H. Ando and S. Wada (this company) for their support and encouragement throughout this work.

Research Laboratory, Teikoku Hormone Mfg. Co., Ltd.
Shimosakunobe, Kawasaki–shi

Hiromu Mori
Kenyu Shibata
Kiyoshi Tsuneda
Masanobu Sawai

Kyoritsu Colledge of Pharmcy
Shibakoen, Minato–ku, Tokyo

Kyosuke Tsuda

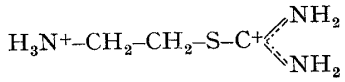
Received May 7, 1968

[Chem. Pharm. Bull. 16(7)1409—1411(1968)]

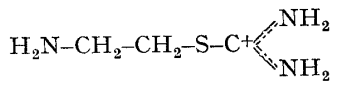
UDC 547.496.3.04 : 541.124

Possibility of the Second-order Reaction concerning the Transguanylation of 2-Aminoethylisothiuronium Salt

2–Aminoethylisothiuronium (AET) salt, a protective agent against a lethal dose of the ionizing radiation,[1] is transguanylated in the physiological condition to 2–mercaptoethylguanidine,[2] which may be an active form of this compound. The rate of the transguanylation is rapid, and the ionized form of AET, which is a reactive species,[3] is half transformed within one minute at 5°.[4] This paper communicated that the half life (τ) of the reaction was inversely proportional to the concentration of the ionized AET.



AET (RNH_3^+)



Ionized AET (RNH_2)

1) R. Shapira, D.G. Doherty, and W.T. Burnett, Jr., *Radiation Res.*, **7**, 22 (1957).
2) J.X. Khym, R. Shapira, and D.G. Doherty, *J. Am. Chem. Soc.*, **79**, 5663 (1957).
3) A. Hanaki, T. Hino, S. and Akaboshi, *Chem. Pharm. Bull.* (Tokyo), **15**, 1446 (1967).
4) A. Hanaki, *Chem. Pharm. Bull.* (Tokyo), submitted.