UDC 547.92.07:542.98

Enzymatic Hydrolysis of Steroidal Esters and its Application to Syntheses of Steroids

Enzymatic hydrolysis of steroidal acetates by a simple procedure employing a commercially available enzyme, diastase,[*1] will be described herein. The hydrolysis is processed in general as follows: Five parts of 2% aqueous solution of diastase is added to one part of 0.2% methanolic solution of a steroidal acetate. After storage at room temperature for 1~10 days, the mixture is extracted with an organic solvent and the extract is evaporated to give an almost pure free alcohol.

A number of steroids possessing an acetoxyl group at various positions were subjected to enzymatic hydrolysis and examined by the paper chromatographic method. As a result it was demonstrated that the diastase shows an interesting selectivity in the hydrolysis depending on the position of acetoxyl group in the steroid. As shown in Fig. 1, the acetoxyl group at positions C-16, C-17,[*2] C-20, and C-21 was hydrolyzed to the free alcohol whereas the other acetates were resistant to the hydrolysis.

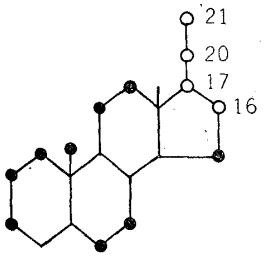


Fig. 1. Selective Hydrolysis of the Acetates by Diastase

○ : Position of the hydrolyzable acetoxyl group
● : Position of the unhydrolyzable acetoxyl group

By use of this selective hydrolysis, 11α,21-diacetoxy-17α-hydroxy-5-pregnene-3,20-dione (I-a), 15α,21-diacetoxy-17α-hydroxy-4-pregnene-3,20-dione (I-b), and 17α-hydroxy-19,21-diacetoxy-4-pregnene-3,20-dione[1] (I-c) were hydrolyzed[*3] to the 11-monoacetate[2] (II-a), 15-monoacetate[3] (II-b), and 19-monoacetate (II-c), m.p. 188~190°, $[\alpha]_D^{23}$ +145° ($CHCl_3$); λ_{max}^{EtOH} 238 mμ (ε 17,000) (*Anal.* Calcd. for $C_{23}H_{32}O_6$: C, 68.29; H, 7.97. Found: C, 67.94; H, 7.94), in substantially quantitative yields. Treatment of (II-c) with tosyl chloride in dimethylformamide yielded the 21-chloro compound (III-c), m.p. 143~146° (decomp.) (*Anal.* Calcd. for $C_{23}H_{31}O_5Cl$: C, 65.32; H, 7.39; Cl, 8.38. Found: C, 65.19; H, 7.47; Cl, 8.29). Dechlorination of (III-c) with sodium iodide in acetic acid followed by saponification with potassium hydrogencarbonate produced 17α,19-dihydroxyprogesterone (IV-c), m.p. 230~235°, $[\alpha]_D^{21}$ +121° (acetone); λ_{max}^{EtOH} 243 mμ (ε 14,300) (*Anal.* Calcd. for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73. Found: C, 72.96; H, 8.73). Similarly, (II-a) was converted via the 21-chloro compound (III-a), m.p. 256~257° (decomp.) (*Anal.* Calcd. for $C_{23}H_{31}O_5Cl$: C, 65.32; H, 7.39. Found: C, 65.22; H, 7.48), into 11α,17α-dihydroxyprogesterone (IV-a).

Enzymatic hydrolysis is also applicable to the ester which is a part of a structure sensitive to acid or alkali (e.g., 16β-acetoxy-17α-hydroxypregnan-20-one). Diastase

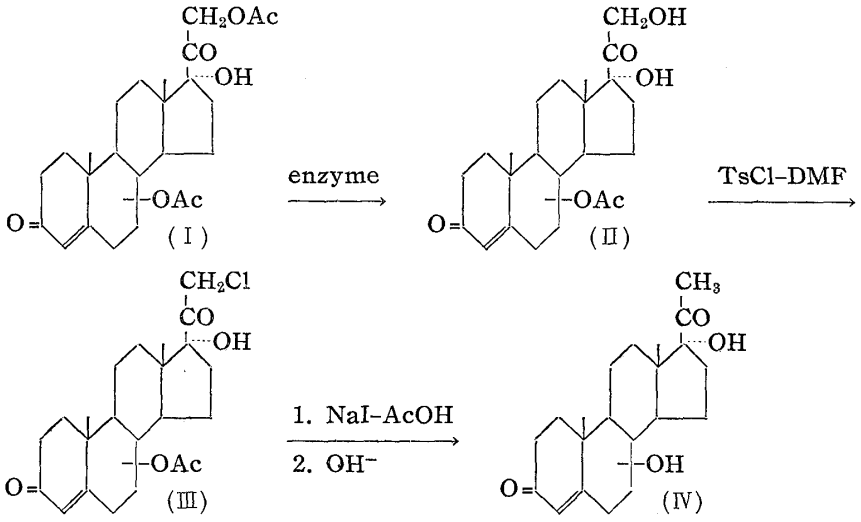
*1 In the present experiment, a commercial diastase (J.P.) supplied by Takeda Pharm. Ind., Ltd. was used. Other diastases, e.g., Taka-diastase and the enzyme obtained from pancreatic gland of the pig are also used with success.

*2 Testosterone acetate (secondary acetoxyl group) is hydrolyzed slowly, but a tertiary acetoxyl group at C-17 is practically inert to hydrolysis.

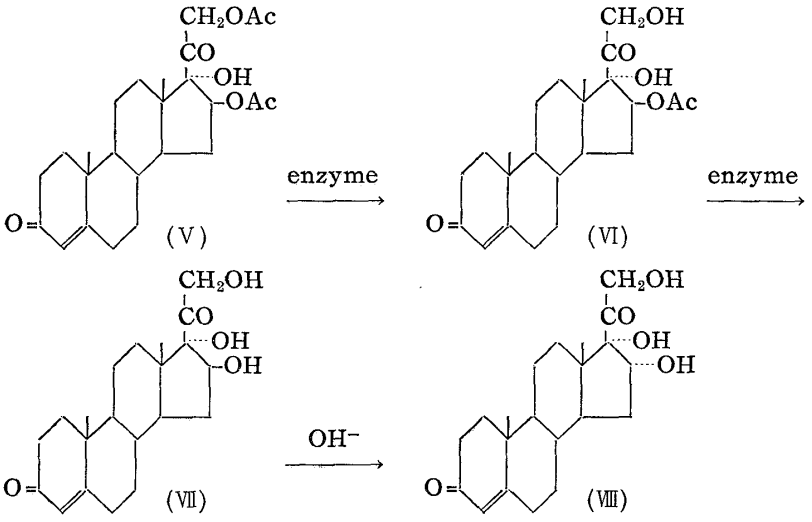
*3 These 21-deacetylations are completed in 1~2 days.

1) M. Nishikawa, H. Hagiwara: This Bulletin, **6**, 226(1958).
2) E.P. Oliveto, *et al.*: J. Am. Chem. Soc., **75**, 3651(1953).
3) K. Tsuda, T. Asai, Y. Sato, T. Tanaka: This Bulletin, **7**, 534(1959).

hydrolysis of 16β,21-diacetoxy-17α-hydroxy-4-pregnene-3,20-dione[4] (V) produced the 16-monoacetoxy compound (VI), m.p. 188~190°, $[\alpha]_D^{21}$ +104°($CHCl_3$); λ_{max}^{EtOH} 240 mμ (ε 15,600) (*Anal.* Calcd. for $C_{23}H_{32}O_6$: C, 68.29; H, 7.97. Found: C, 68.61; H, 7.92), and the free steroid*4 (VII), m.p. 190~191°, $[\alpha]_D^{21}$ +118°($CHCl_3$); λ_{max}^{EtOH} 241 mμ (ε 16,300) (*Anal.* Calcd. for $C_{21}H_{30}O_5$: C, 69.58; H, 8.34. Found: C, 69.56; H, 8.11). (VII) was isomerized by alkali into the 16α-epimer (VIII).[5]



(I), (II), (III) (a: 11α-OAc, b: 15α-OAc, c: 19-OAc),
(IV) (a: 11α-OH, c: 19-OH)



Oxidation of (VI) with sodium bismuthate yielded 16β-acetoxy-4-androstene-3,17-dione (IX), m.p. 167~168°, $[\alpha]_D^{21}$ +152° ($CHCl_3$); λ_{max}^{EtOH} 239 mμ (ε 15,800) (*Anal.* Calcd. for $C_{21}H_{28}O_4$: C, 73.22; H, 8.19. Found: C, 72.79; H, 8.09). Subsequent rearrangement of the latter

4) (V) is derived from 16α,17α-epoxy-21-acetoxy-4-pregnene-3,20-dione by treatment of the latter with sulfuric acid and acetic acid (K. Heusler, A. Wettstein: Chem. Ber., **87**, 1301(1954)).

*4 (VI) is the main hydrolysis product after storage of the reaction mixture for 20 hours and (VII) for 10 days.

5) Bernstein, *et al.* (S. Bernstein, M. Heller, S.M. Stolar: J. Am. Chem. Soc., **81**, 1256(1959)) first discovered that (V) epimerizes into (VIII) by alkali. Thereafter Kuo, *et al.* (H. Kuo, D. Taub, N.L. Wendler: Chem. & Ind. (London), **1959**, 1128) presented a plausible mechanism for this epimerization, in which they supposed 16β-hydroxy compound such as (VII) to be a possible intermediate although it was not isolated by these authors.

with sulfuric acid in methanol[6] afforded 16-oxotestosterone.[7]

Further studies on the hydrolysis of the esters possessing other acyloxyl groups and other aspects of this enzyme reaction will be reported in detail in a later publication.

The authors are greatly indebted to Director Dr. S. Kuwada and Dr. T. Matsukawa, Head of 1st Division, of these Laboratories, for their encouragement throughout this work.

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6) W. S. Johnson, B. Gastambide, R. Pappo: J. Am. Chem. Soc., **79**, 1991(1957).
7) A. S. Meyer, M. C. Lindberg: *Ibid.*, **76**, 3033(1954); W. J. Adams, *et al.*: J. Chem. Soc., **1956**, 297.

UDC 547.918:582.938

On the Isolation and Structure of Penupogenin

The isolation of an aglycone, cynanchogenin, from the root of *Cynanchum caudatum* MAX.[1] was reported previously and the structure (A) was given for it.[2,3] In this communication, isolation of another aglycone, penupogenin, from the same root and its structure are described.

The aglycone mixture, obtained by the hydrolysis of the crude glycoside from the root,[1] was chromatographed over alumina column and the mixture separated into two fractions. The first mainly consisted of cynanchogenin. The second, which gave green Lieberman-Burchard reaction, was rechromatographed over alumina column. The eluate with 1% MeOH-$CHCl_3$ gave needles from ether-petroleum ether. Further recrystallizations from ether gave white needles, m.p. 145~150°, and this product was named penupogenin*1 (II).

Penupogenin shows green color with Lieberman-Burchard reaction, orange→violet with conc. H_2SO_4, pink→greyish blue with $SbCl_3$, and negative Keller-Kiliani reaction. UV λ_{max}^{EtOH} 279 mμ (ε 22,000); IR λ_{max}^{KBr} cm^{-1}: 3400 (OH), 1690 (C=O conj.), 1630 (C=C conj.), 1580, 1600 (aromatic). These data strongly suggest that penupogenin is a cinnamoyl ester.

Hydrolysis of penupogenin gave an acid substance (III), m.p. 136°, and a neutral substance (IV), m.p. 260°/150° (from MeOH). The acid substance (III) gave only one spot on the chromatogram and was identified as cinnamic acid by the mixed m.p. (IV) gave much the same color reactions as (II) and was expected to have the formula $C_{21}H_{34}O_6$ (*Anal.* Calcd.: C, 65.94; H, 8.96. Found: C, 65.66; H, 7.78). Acetylation of (IV) afforded a triacetate, m.p. 205°, $C_{27}H_{40}O_9$ (*Anal.* Calcd.: C, 63.76; H, 7.59. Found: C, 63.45; H, 7.58).

In 1939, Cornforth, *et al.*[4] isolated sarcostin, $C_{21}H_{34}O_6$, from an Australian Asclepiadaceae plant, *Sarcostemma australe* R. BR. and Reichstein, *et al.*[6] also isolated the same

*1 "Penup" is one of the Ainu names for *Cynanchum caudatum* MAX.
1) H. Mitsuhashi, Y. Shimizu: This Bulletin, 8, 313(1960).
2) *Idem*: *Ibid.*, 8, 318(1960).
3) *Idem*: *Ibid.*, 7, 749, 949(1959).
4) J. W. Cornforth, *et al.*: J. Chem. Soc., **1939**, 737; **1940**, 1443.
5) T. Reichstein, *et al.*: Helv. Chim. Acta, **42**, 1014(1959).
6) J. W. Cornforth: Chem. & Ind. (London), **1959**, 602.