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8β-HYDROXYLATION OF REICHSTEIN'S SUBSTANCES BY MICROORGANISM

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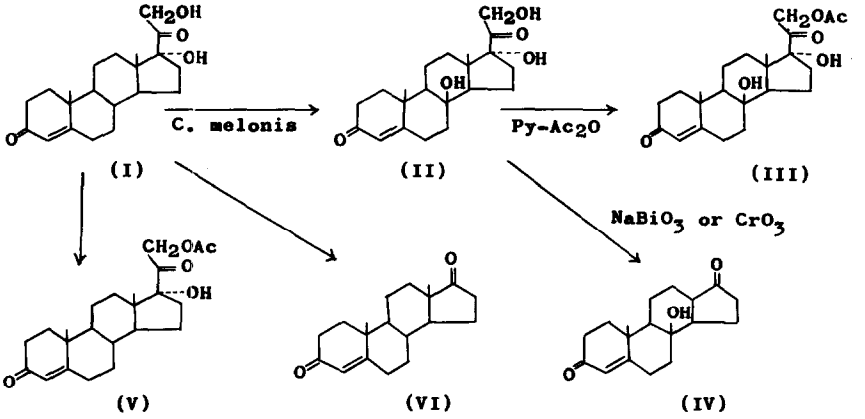
IN a previous paper[1] on hydroxylation of Reichstein's substance S (I) by microorganisms, it was reported that a new tertiary hydroxylated derivative (II), which was assumed to be an 8β-hydroxyl derivative, was obtained from I in good yields by the use of Cercospora melonis [Corynespora melonis (cke) Lindau].

Compound II was obtained as colorless needles (m.p. 225-227°C, $[\alpha]_D^{24}$ +115°),[2] which showed a positive color test with alkaline triphenyl tetrazolium chloride and infrared absorption bands of Δ^4-3-ketone and 20-ketone. Acetylation of II gave a monoacetate (III, m.p. 211-212°C, $[\alpha]_D^{24}$ +145°). The infrared and NMR spectra of III showed only the presence of a 21-acetoxyl group. A monohydroxy-4-androstene-3,17-dione (IV, m.p. 233-235°C, $[\alpha]_D^{24}$ +193°) was derived from II by oxidation with sodium bismuthate in 50% aqueous acetic acid. Attempts to acetylate IV were unsuccessful. Furthermore, IV was also obtained from II by chromium trioxide

[1] E.Kondo, K.Morihara, Y.Nozaki and E.Masuo, J. Agr. Chem. Soc. Japan 34, 844 (1960).

[2] Analysis of all the compounds described corresponded to the calculated values.

oxidation in acetic acid. No signal of the proton attached to the hydroxyl-bearing carbon atom was found in the NMR spectrum of III or IV. Accordingly, the newly introduced hydroxyl group may be tertiary.



Recently, Hayano, et al.[3] showed that the hydroxyl group introduced microbiologically has the same configuration as the hydrogen replaced. Therefore, the tertiary hydroxyl group in II could be at either the 8β-, 9α- or 14α-position. However, II was neither identical with 14α,17α,21-trihydroxy-4-pregnene-3,20-dione[4,5] nor 9α,17α,21-trihydroxy-4-pregnene-3,20-dione.[5,6] These facts

[3] M.Hayano, M.Gut, R.I.Dorfman, O.K.Sebek and D.H.Peterson, J. Amer. Chem. Soc. 80, 2336 (1958).

[4] E.Kondo, J. Agr. Chem. Soc. Japan 34, 762 (1960).

[5] E.Kondo and T.Mitsugi, J. Agr. Chem. Soc. Japan 35, 521 (1961).

[6] The microbiological 9α-hydroxylation has been clearly established by Dodson and Muir[7] and Schubert, et al.[8] The 8β- or 9α-hydroxylations previously reported by a few authors[9] are at present considered as 9α-hydroxylations.

[7] R.M.Dodson and R.D.Muir, J. Amer. Chem. Soc. 80, 6148 (1958).

[8] A.Schubert, D.Onken, R.Siebert and K.Heller, Chem. Ber. 91, 2549 (1958).

[9] D.Stone, M.Hayano, R.I.Dorfman, O.Hechter, C.R.Robinson and C. Djerassi, J. Amer. Chem. Soc. 77, 3926 (1955); J.Fried, R.W.Thoma, D.Perlman, J.E.Herz and A.Borman, Recent Progr. Hormone Res. 11, 149 (1955); S.H.Eppstein, P.D.Meister, D.H.Peterson, H.C.Murray, H.M.Leigh Osborn, A.Weintraub, L.M.Reiceke and R.C.Mecks, J. Amer. Chem. Soc. 80, 3382 (1958).

suggest 8β as the most preferred position for the tertiary hydroxyl group.

In NMR studies on steroids, signal shifts of the angular methyl groups due to the effects of various substituents have been frequently reported.[10,11,12,13,14] Recently, this effect as produced by a hydroxyl group has been examined by Kawazoe, et al.,[15] who concluded that a remarkable downfield shift of the signal peak of angular methyl groups is caused by a hydroxyl group that has a 1,3-diaxial relationship to the methyl group in a chair-formed cyclohexane ring.

For comparison, various hydroxyl derivatives of Reichstein's substance S 21-acetate (V) and 4-androstene-3,17-dione (VI) were examined. Chemical shifts of main signal peaks of the derivatives of V and VI are shown in Tables I and II,[16] respectively. Effects of the hydroxyl groups[17] in the various positions derived from Tables I and II are shown in Table III, the last column of which shows those effects observed in other steroids. As seen in Table III only the hydroxyl group in III or IV, besides the 11β-hydroxyl group, has

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[10] J.N.Shoolery and M.T.Rogers, J. Amer. Chem. Soc. 80, 5121 (1958).

[11] J.S.G.Cox, E.O.Bishop and R.E.Richards, J. Chem. Soc. 5118 (1960).

[12] G.Slomp,Jr. and B.R.McGarvey, J. Amer. Chem. Soc. 81, 2200 (1959).

[13] R.F.Zürcher, Helv. Chim. Acta 44, 1380 (1961).

[14] J.C.Jaquesey, J.M.Lehn and L.Levisalles, Bull. soc. chim. France 2444 (1961).

[15] Y.Kawazoe, Y.Sato, M.Natsume, H.Hasegawa, T.Okamoto and K.Tsuda, Chem. & Pharm. Bull. 10, 338 (1962).

[16] All the spectra were taken with a Varian model A-60 analytical NMR spectrometer system on 2-3% solutions in chloroform containing tetramethylsilane as an internal reference.

[17] The substituent effect on the chemical shifts of the 18- and 19-methyl groups is represented by the difference in the chemical shifts between the methyl groups in a hydroxyl derivative and those in its parent steroid.

Table I

Chemical shifts of main signals in the NMR spectra of hydroxyl derivatives of Reichstein's substance S 21-acetate (V). (τ)

Compounds			19-H	18-H	21-OAc	21-H[a]	4-H
Reichstein's substance S 21-acetate (V)			8.82	9.28	7.83	5.09 4.90	4.25
2β-Hydroxy-	"	c	8.63	9.28	7.83	5.09 4.90	4.17
6β-Hydroxy-	"	c,d	8.60	9.23	7.82	5.17 4.99	4.17
9α-Hydroxy-	"	d	8.67	9.27	7.83	5.07 4.93	4.22
11β-Hydroxy-	"	b,c,d	8.56	9.03	7.83	5.15 5.01	4.33
14α-Hydroxy-	"	c,d	8.77	9.17	7.82	5.09 4.90	4.23
8β-Hydroxy-	"	(III)	8.66	9.02	7.83	5.21 4.85	4.22

a: quartet of AB system, J = 17.5 cps.
b: ref. (1) c: ref. (4) d: ref. (5)

Table II

Chemical shifts of main signals in the NMR spectra of hydroxyl derivatives of 4-androstene-3,17-dione (VI). (τ)

Compounds			19-H	18-H	4-H
4-Androstene-3,17-dione (VI)			8.79 8.76[b]	9.08 9.06[b]	4.31
6β-Hydroxyl-	"	b	8.58[b]	9.02[b]	
9α-Hydroxyl-	"	a	8.65	9.08	4.11
11β-Hydroxyl-	"		8.52 8.50[b]	8.82 8.82[b]	4.30
14α-Hydroxyl-	"	a	8.79 8.76[b]	8.95 8.94[b]	4.23
8β-Hydroxyl-	"	(IV)	8.63	8.82	4.20

a: ref. (5) b: ref. (15)

Table III

Substituent effect of hydroxyl group (ppm)

Site of hydroxyl group	Difference from parent compound				Results in other steroids	
	Reichstein's substance S 21-acetate (V)		4-Androstene-3,17-dione (VI)			
	19-H	18-H	19-H	18-H	19-H	18-H
2β	-0.19	0.00	-	-	-0.24[a]	0.00[a]
6β	-0.22	-0.05	-0.18[b]	-0.04[b]	-0.18[b] -0.225[c]	-0.07[b]
9α	-0.15	-0.01	-0.14	0.00	-	-
11β	-0.26	-0.25	-0.27[b] -0.26[b]	-0.26[b] -0.24[b]	-0.25[b] -0.258[c]	-0.24[b]
14α	-0.05	-0.11	0.00[b] 0.00[b]	-0.13[b] -0.12[b]	-0.005[b]	-0.11[b]
14β	-	-	-	-	-0.025[c] -0.01[d]	+0.09[d]
15β	-	-	-	-	-0.03[b]	-0.27[b]
8β	-0.16	-0.26	-0.16	-0.26	-	-

a: K.Tori, T.Komeno and S.Nakashima, unpublished results,
b: ref. (15), c: ref. (13), d: K.Tori and H.Ishii, unpublished results.

marked effects upon both angular methyl groups. This fact implies that the hydroxyl group in III or IV is situated at a 1,3-diaxial position to both the 18- and 19-methyl groups. Thus, the only probable position for the tertiary hydroxyl group is 8β. Since the spatial relationship of the 19-methyl group to the 8β-hydroxyl group is similar to that to the 6β-hydroxyl group as seen from examination on Dreiding models, the 8β-hydroxyl group influences the 19-methyl signal to the same extent as does the 6β-hydroxyl group. Such similarity is seen also in the relationships of the 18-methyl group to the 8β- and to the 11β-hydroxyl group.

The new hydroxyl group could be located at the 8α-, 9β- or 14β-position, if this hydroxylated steroid were of an unnatural configuration, although Hayano, et al.[3] have demonstrated that such a

configuration is highly improbable. However, the 14β-position can be excluded as seen from the examples in the last column of Table III. It is almost certain that the 9β-hydroxyl group has little effect upon the 18-methyl signal. The remaining possibility of 8α-substitution may be eliminated because the 8α-hydroxyl group can hardly influence the angular methyl groups directly. Even bearing in mind the closer proximity of the two angular methyl groups which is brought about by the transformation of the B—C ring juncture, as seen from Dreiding models, and the consequent effect of the groups on each other, this effect is not so pronounced.[13]

Consequently, the hydroxyl group in III or IV, and accordingly in II, can be established to be the 8β-substituent.

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