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# THE ENZYMATIC HYDROXYLATION OF STEROIDAL β-DIKETONES

Saul L. Neidleman, Patrick A. Diassi, Barbara Junta,
Raymond M. Palmere, Paul Grabowich and Samuel C. Pan

The Squibb Institute for Medical Research

New Brunswick, New Jersey

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The enzymatic hydroxylation of various aromatic compounds and proline have been reported[1-3] using horseradish peroxidase. However, only one example of a steroidal hydroxylation, viz., the 10β-hydroxylation of 17α-ethynyl-17β-hydroxyestr-5(10)-en-3-one using this enzyme system has been recorded[4]. We would like to report the facile hydroxylation of 16-keto-progesterone (II)[5] and 16-keto-A-norprogesterone (VI) by similar enzyme systems and by a non-enzymatic method.

Both 16-ketoprogesterone (II) and 16-keto-A-norprogesterone (VI) were prepared by the Jones oxidation[6] of 16α-hydroxy-progesterone[7] (I) and 16α-hydroxy-A-norprogesterone[8] (V) respectively. Like II[5], 16-keto-A-norprogesterone, m.p. 128-130°; $[\alpha]_D^{22}$-44° (chloroform) exists mainly in the enol form as evidenced by its ultraviolet absorption maxima at 233 and 286 mμ, infrared absorption bands at 1717, 1654 and 1625 cm.$^{-1}$[9] and NMR spectrum which has a

hydroxyl proton resonance at -3.77$\tau$ indicating strong hydrogen bonding[10].

Reaction of II and VI with horseradish peroxidase, glucose and glucose oxidase in a phosphate buffer (pH 6.0) at room temperature for two hours gave 30% yields of the 17α-hydroxylated products III, m.p. 202-205°; $[\alpha]_D^{25}$ -90.7° (chloroform), and VII, m.p. 218-220°, $[\alpha]_D^{22}$ -204° (chloroform), respectively, based on elemental analysis[12] and the following: The ultraviolet spectra [$\lambda_{max.}^{alc.}$ 239 mμ ($\varepsilon$, 18700) for III; $\lambda_{max.}^{alc.}$ 232 ($\varepsilon$, 18700) for VII] no longer showed evidence for enols and were unchanged on addition of mild alkali. The infrared spectra ($\lambda_{max.}^{CCl_4}$ 3550, 3450, 1756, 1708 and 1681 cm.$^{-1}$ for III and $\lambda_{max.}^{CCl_4}$ 3550, 3400, 1753, 1704, and 1686 cm.$^{-1}$ for VII) showed two strongly bonded hydroxyl stretching frequencies in each case which could be assigned to the 17-hydroxyl bonded to either the C-16 carbonyl or C-20 carbonyl[13]. The NMR spectra (Table I) were consistent with the assigned structures and also showed bonded hydroxyl protons at 5.78$\tau$ for III and 6.17$\tau$ for VII.

That this hydroxylation reaction was enzymatic was evident from the fact that when the horseradish peroxidase was omitted from the reaction mixture no reaction occurred. Hydrogen peroxide could be substituted for the glucose-glucose oxidase.

The ketols III and VII could also be obtained in 60% yield by reaction of II and VI with m-chloroperbenzoic acid followed by treatment with dilute alkali. This latter reaction together with the NMR data supports the 17α- assignment for the hydroxyl group and indeed on treatment with alkali at room temperature

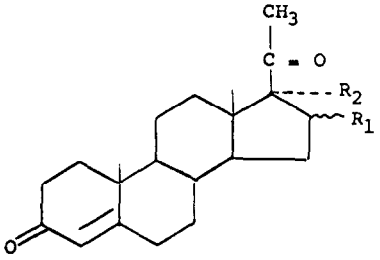
they were rearranged [14] to the ring D lactones IV m.p. 205-207°; $[\alpha]_D^{22}$ +3.6° (chloroform), and VIII, m.p. 170-172°; $[\alpha]_D^{25}$ -105° (chloroform).

TABLE I

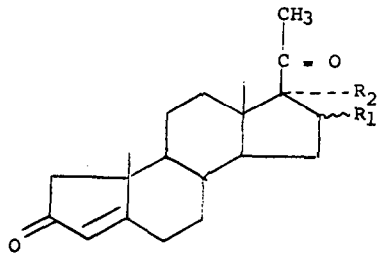
Chemical Shifts ($\tau$) for 16,20-Diketo Steroids

| Compound | 18-$CH_3$ | 19-$CH_3$ | 21-$CH_3$ | 17-OH | 17α-H |
|---|---|---|---|---|---|
| II | 8.93 | 8.76 | 7.99 | | |
| III | 9.01 | 8.78 | 7.73 | 5.78 | |
| IV | 9.01 | 8.81 | 7.71 | | 5.75 |
| VI | 8.90 | 8.77 | 7.98 | | |
| VII | 9.03 | 8.78 | 7.72 | 6.17 | |
| VIII | 9.00 | 8.83 | 7.71 | | 5.74 |

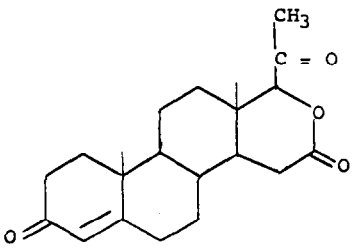
When the enzymatic conditions were applied to 15-keto-androstenedione[15] a more polar product could be detected by TLC but could not be isolated. Progesterone and 16α-hydroxy-progesterone showed no evidence of hydroxylation under the enzymatic conditions.



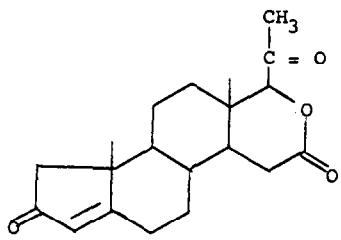
| | $R_1$ | $R_2$ |
|---|---|---|
| I |  | H |
| II | =O | H |
| III | =O | OH |



| | $R_1$ | $R_2$ |
|---|---|---|
| V |  | H |
| VI | =O | H |
| VII | =O | OH |



IV



VIII

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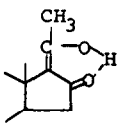
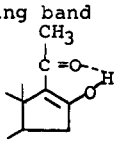
9. No hydroxyl stretching band was visible in the infrared but it may be submerged beneath the C-4 stretching band at 3000-2850 cm.$^{-1}$.

10. Of the two most likely enols,  and 

i ii

the NMR indicates that in deuteriochloroform at room temperature the equilibration of the various tautomers is sufficiently rapid that the 21-methyl bands appears as a singlet (cf. Table I) and the chemical shift indicates[11] that the 16-keto-$\Delta^{17(20)}$-tautomer is the predominant form.

11. The chemical shifts for the 21-methyl protons of progesterone and 16-dehydroprogesterone are at 7.87 and 7.73$\tau$, respectively, whereas the chemical shift for a vinyl methyl is 8.1-8.3$\tau$.

12. Satisfactory analyses were obtained for all new compounds described herein.

13. 17α-Hydroxyprogesterone shows a bonded hydroxyl (3500 $cm.^{-1}$) and a non-bonded hydroxyl (3611 $cm.^{-1}$) in the infrared.

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