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MASS SPECTROMETRY OF STEROID SYSTEMS—V*

DETERMINATION OF THE CONFIGURATION OF SECONDARY STEROID ALCOHOLS BY MASS SPECTROMETRY

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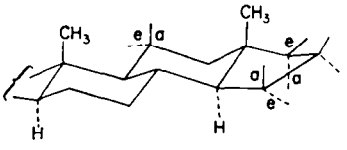
Abstract—The mass spectra of epimeric secondary alcohols of the progesterone series taken by direct admission of the sample into the ion source have been studied. It was shown that the mass spectra of alcohols with the axial hydroxyl differ sharply from those of alcohols with the equatorial hydroxyl by the intensity ratio of the M—H_2O peak to the M^+ peak (for a-OH group $M-H_2O/M^+ > 1$, whereas for e-OH group $M-H_2O/M^+ < 1$). This makes the determination of the configuration of the corresponding alcohol in the monohydroxy steroid series possible on the basis of its mass spectrum even in the case when its second epimer is absent.

RECENTLY[1] using two pairs of epimeric tertiary alcohol derivatives of $\Delta^{8(9)}$-D-homo-estradiol-3,17a it was shown that compounds of this series with the axial OH group readily undergo dehydration under electron impact. The corresponding epimers with the equatorial hydroxyl dehydrate less readily under these conditions.

Continuing work on the mass spectrometry of steroid systems was made a study of the mass spectra of a number of the epimeric secondary alcohols with the OH group both in a six-membered ring, 11α(I)- and 11β(II)-hydroxyprogesterones and in a five-membered ring, 16α(III)-, 16β(IV)-, 15α(VI)-, 15β(VII)-hydroxyprogesterones, 11α,16α-dihydroxyprogesterone (V) and 15α-hydroxy-Δ^4-androsten-3,17-dione (VIII).



The first report on the determination of the configuration of secondary cyclic alcohols and their acetates by mass spectrometry was given by Biemann and Seibl.[2] But the mass spectra of the epimers (measured by evaporation of the sample in a hot metallic system) could not be distinguished from each other (the ratio $M-H_2O/M^+$ value changing insignificantly on passing from the alcohols with a-OH to those with e-OH

* For paper IV see N. S. Wulfson, V. I. Zaretskii, V. L. Sadovskaya, A. V. Semenovsky, W. A. Smit and V. F. Kucherov, *Tetrahedron.*

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[1] V. I. Zaretskii, N. S. Wulfson, V. G. Zaikin, S. N. Ananchenko, V. N. Leonov and I. V. Torgov, *Tetrahedron* **21**, 2769 (1965)

[2] K. Biemann, J. Seibl, *J. Amer. Chem. Soc.* **81**, 3149 (1959).

group). By using the glass system with direct admission of the specimen into the ion source near the ionization chamber we succeeded in obtaining the low temperature mass spectra of the epimers I–VIII (145–165°, i.e. the temperature used was considerably lower than the m.p. of the examined compounds). The mass spectra of α-hydroxysteroids taken under such conditions differ considerably from those of corresponding β-hydroxy epimers mainly by the intensity ratio of the $M-H_2O$ peak to the M^+ peak. For instance, the mass spectra of the compounds with axial hydroxyl reveal the $M-H_2O$ peak of much greater intensity than that of M^+ peak whereas in the case of their e-OH epimers the reverse ratio occurs. Consequently the configuration of the corresponding alcohol in the monohydroxy steroid series may be determined on the basis of its mass spectrum even if the second epimer is not available.

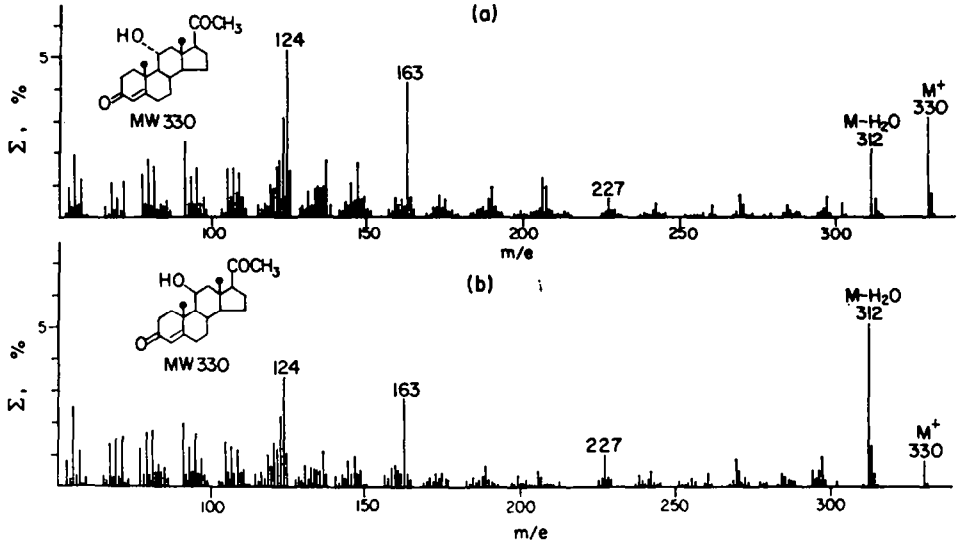


FIG. 1. Mass spectra of: (a) 11α-Hydroxyprogesterone (I)
(b) 11β-Hydroxyprogesterone (II).

11α(I)- *and* 11β(II)[3]-*Hydroxyprogesterones.* A comparison of the mass spectra of compounds I and II shows that (analogously to the tertiary alcohols of the D-homo series[1]) 11β-hydroxyprogesterone (II) with the axial hydroxyl readily eliminates a molecule of water (Fig. 1 and Table 1) whereas the molecular ion of 11α-epimer (I) with the equatorial OH group is much more stable under these conditions and dehydrates less readily. The difference in the ease of dehydration under electron impact was observed by Biemann[2] in the case of epimeric 3-hydroxy steroid (androsterone and epiandrosterone). The $M-H_2O/M^+$ ratio value was found greater for the isomer with axial 3-OH group.

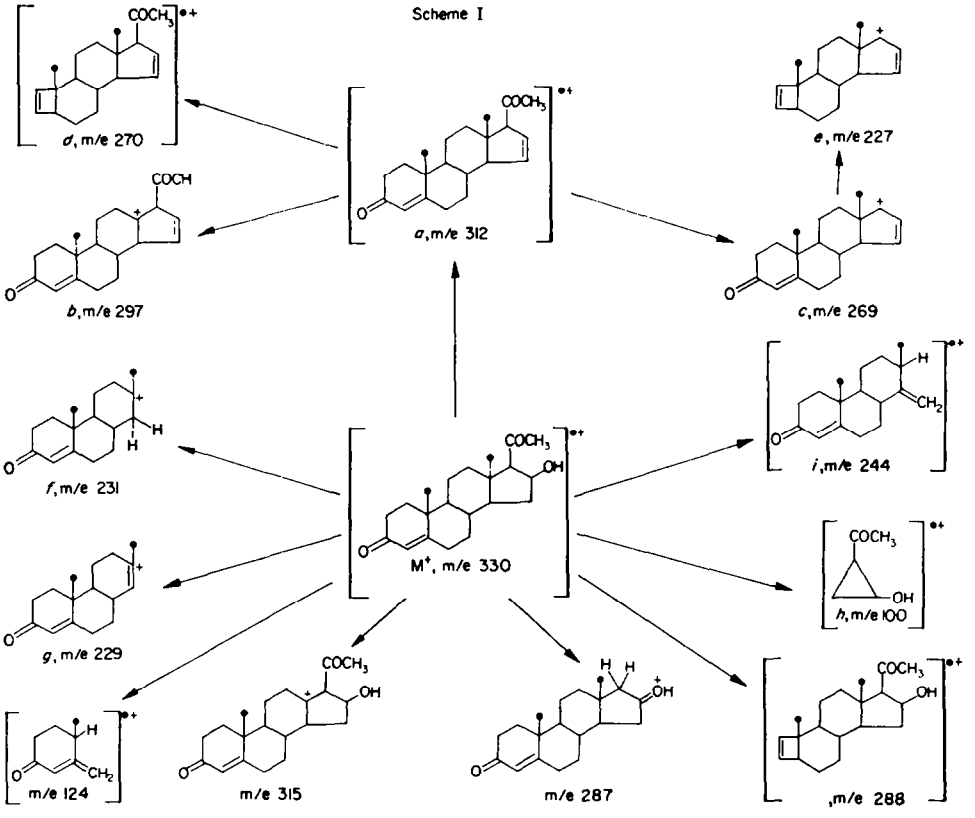
16α(III)- *and* 16β(IV)-*Hydroxyprogesterones.* The mass spectra of III and IV differ essentially from each other. There is an intense molecular ion peak in the spectrum of IV (β-hydroxy) and rather small $M-H_2O$ peak. The molecular peak in the case of the α-epimer (III) is insignificant and the $M-H_2O$ peak is the most abundant one in the spectrum. That is why the intensity of the fragment peaks at m/e 297 (*b*),

[3] Leonid M. Kogan, I. V. Ulezlo, I. K. Kozlova, N. N. Suvorov, S. L. Portnova, G. K. Skrjabin and I. V. Torgov, *Izv. Akad. Nauk SSSR, Otd. Chim. Nauk* 2008 (1964).

TABLE 1. ABUNDANCE OF CHARACTERISTIC PEAKS (% OF TOTAL IONIZATION) IN THE MASS SPECTRA OF HYDROXYPROGESTERONES I–IV AND VI–VIII

Compound	I	II	III	IV	VI	VII	VIII
Substituent	11α	11β	16α	16β	15α	15β	15α
M^+	3·2	0·9	0·08	2·2	7·6	1·0	7·6
$M-H_2O$	2·1	5·1	3·9	0·7	1·6	4·9	0·5
$\frac{M-H_2O}{M^+}$	0·66	5·7	49	0·32	0·21	4·9	0·07
m/e 231	—	—	2·7	0·3	0·7	3·0	—

Scheme I



269 (*c*), 270 (*d*) and 227 (*e*) resulting from degradation of the ion at m/e 312 (*a*) (Fig. 2 and Scheme 1) increases on passing from β(IV)- to α(III) epimer. The difference in the degree of dehydration in 16α- and 16β-hydroxyprogesterones supports the conclusion that the α- and β-linkages at C_{16} atom resemble in character the axial and equatorial bonds respectively.

The characteristic feature of the mass spectrum of 16α-hydroxyprogesterone (III) is an intense peak at m/e 231 (fragment *f*) which is practically absent in the case of

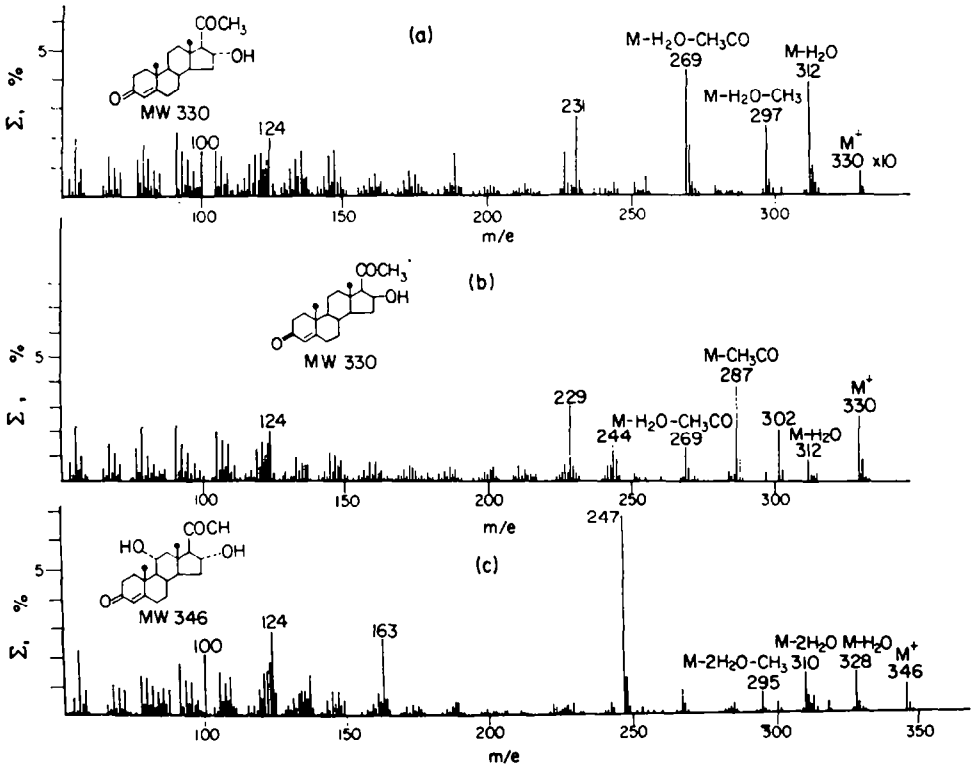
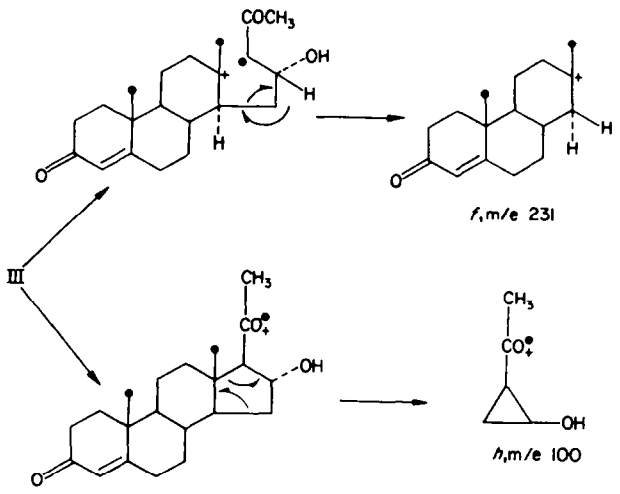


FIG. 2 Mass spectra of: (a) 16α-Hydroxyprogesterone (III) (b) 16β-Hydroxyprogesterone (IV) (c) 11α,16α-Dihydroxyprogesterone (V).

β-epimer (IV). At the same time the mass spectrum of the latter reveals the tricyclic fragment peak at m/e 229 (*g*) which is the most common species found in the spectra of almost all steroid systems.[4] The fragment *f* contains rings A, B and C (tricyclic fragment also), which is confirmed by the shift of the corresponding peak m/e by 16 mass units (to m/e 247) in the spectrum of 11α,16α-dihydroxyprogesterone (V), and is formed as a result of the fissions of the 13–17 and 14–15 bonds accompanied by a hydrogen atom transfer to the charged fragment (Scheme 2). Cleavage of the same bonds accompanied by a charge localization on the fragment containing C_{15}, C_{16}, C_{17}, C_{20} and C_{21} atoms gives rise to the m/e 100 ion (*h*). The m/e 100 peak is prominent in the spectrum of 16α-hydroxyprogesterone (III) and is practically absent in the case of β-epimer (IV). The structure of fragment *h* is confirmed by comparison with the mass spectra of 16α-OD derivative III and 15-hydroxyprogesterones. In the former the corresponding peak is shifted by a unit (to m/e 101), whereas in the latter case the mass number of this peak remains unaffected. It might have been supposed that a hydrogen atom of the 16-OH group would take part in the formation of the ion *f* (m/e 231) as it was noted in the case of tertiary alcohols.[1] However, the m/e 231 peak is only shifted to the m/e 232 peak to the extent of 24% as observed in the mass spectrum of 16α-OD analogue III. In all probability the process of the ion formation also involves the transfer of a hydrogen atom from C_{16} and therefore the greater intensity

[4] L. Peterson, *Analyt. Chem.* **34**, 1781 (1962); N. S. Wulfson, V. I. Zaretskii, V. G. Zaikin, G. M. Segal, I. V. Torgov and T. P. Fradkina, *Tetrahedron Letters* 3015 (1964).

Scheme 2.



of the m/e 231 peak in the case of 16α-hydroxy epimer than in the spectrum of its β-analogue may be connected with the fact that a 16β-hydrogen migrates more easily than a 16α-hydrogen.

15α(VI)- *and* 15β(VII)-*Hydroxyprogesterones.* Contrary to the mass spectra of isomeric 16-hydroxyprogesterones (III and IV) (where the greater value of the $M-H_2O/M^+$ ratio is characteristic for α-isomer), in the case of 15α- and 15β-hydroxyprogesterones a reverse regularity is found out (Fig. 3a, b). It is explained by the fact that the OH group of 15α- and 15β-hydroxyprogesterones possesses correspondingly an equatorial and axial character. An analogous picture is observed in the mass spectrum of 15α-hydroxy-Δ^4-androsten-3,17-dione (VIII) in which the intensity of the M-18 peak only is 20% from the molecular ion intensity which is predominant in the spectrum. The main features different in the mass spectrum of 15β-hydroxyprogesterone (VII) as well as its 16α-hydroxy analogue (III) are the considerable intensity of the fragments *f* (m/e 231) and *h* (m/e 100) peaks. The mechanism of their formation may be similar to that described for III. At the same time the intensities of the m/e 231 and 100 peaks are insignificant in the mass spectra of 15α-hydroxyprogesterone (VI) and 15α-hydroxy-Δ^4-androsten-3,17-dione (VIII) (Table 1 and Fig. 3a, c).

As no epimeric 17-hydroxy steroids were available, it is worth mentioning that the steroids of the 17β-hydroxy series (estradiol, 19-nor-testosterone) in which the OH group has an equatorial character analogous to 15α- and 16β-hydroxyprogesterones dehydrate less readily upon electron bombardment.*

It is to be noted that there are characteristic peaks identifying 11, 15 and 16-hydroxyprogesterones in the mass spectra of alcohols I–VII. In fact, the spectra of 16-hydroxy compounds (III and IV) have peak at m/e 244 (fragment *i*), which is absent in the case of the 15-hydroxy derivatives (VI and VII) (Figs. 2 and 3). This fragment is formed directly from the molecular ion of substances III and IV as a result of the abstraction C_{16} and C_{17} atoms with the attached substituents (Scheme 1).

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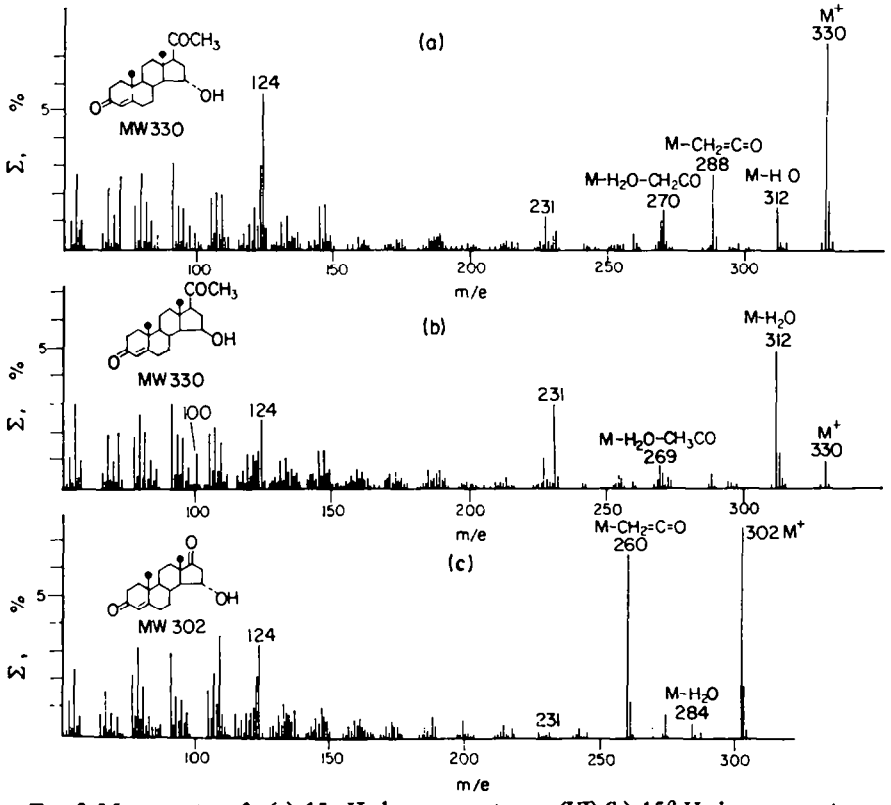

FIG. 3 Mass spectra of: (a) 15α-Hydroxyprogesterone (VI) (b) 15β-Hydroxyprogesterone (VII) (c) 15α-Hydroxy-Δ^4-androsten-3,17-dione (VIII).

The mass spectra of 15- and 16-hydroxyprogesterones differ from those of their 11-hydroxy analogues by the presence of the peak at m/e 100 (ion *h*) in the former (Scheme 1). On the other hand, 11-hydroxyprogesterones show a peak at m/e 163, which is absent in the spectra of III–IV and VI–VII. The fragmentation patterns characteristic for 11- and 16-hydroxyprogesterones can be observed in the case of 11α,16α-dihydroxyprogesterone (V). Therefore, the method of mass spectrometry may be used for the determination of the hydroxyl group position in dihydroxy-steroid series as well.

16β-Hydroxyprogesterone (IV) was prepared from 16α,17α-epoxyprogesterone as follows: ketalization of 16α,17α-epoxyprogesterone led to 3,20-bisethylenedioxy-16α,17α-epoxy-Δ^5-pregnen (IX). Reduction of IX by sodium in ethanol solution[5] gave 3,20-bisethylenedioxy-Δ^5-pregnen-16α-ol (X). Oxidation of X by Sarett reagent[6] with subsequent reduction and hydrolysis yielded IV (cf. 7).

11α,16α-Dihydroxyprogesterone (V) was prepared by fermentation of 11α-hydroxyprogesterone (I) with *Streptomyces roseochromogenus* ATCC 3347. This culture is known[8] to 16α-hydroxylate of a wide range of steroids.

[5] B. Camerino, C. G. Alberti, *Gazz. Chim. Ital.* **85**, 56 (1955).
[6] G. I. Poos, G. E. Arth, R. E. Beyler and L. M. Sarett, *J. Amer. Chem. Soc.* **75**, 422 (1953).
[7] S. Bernstein, M. Heller and S. M. Stolar, *J. Amer. Chem. Soc.* **77**, 5327 (1955).
[8] C. E. Holmlund, L. I. Feldmann, R. H. Blank, N. Barbacci and B. Nielsen, *Sci. Rept. Inst. Super. Sanita* **1**, 289 (1961).

EXPERIMENTAL

The mass spectra were taken on the commercial instrument MX-1303, furnished with a glass system with direct admission of the sample into the ion source near the ionization chamber and a stabilization of temp. at ionizing energy 70 eV and temp of 145–165° (±1°).

Preparation of 16β-*hydroxyprogesterone* (IV). Compound IX (m.p. 182–184°; lit. values[9]: m.p. 183·5–185°) obtained by ketalization of 16α,17α-epoxyprogesterone, was reduced with Na in EtOH[5] to X. The alcohol X without further purification was oxidized by Sarett reagent[6] to XI. Reduction of the ketone XI with $LiAlH_4$ and subsequent hydrolysis afforded IV, m.p. 199–205°, IR spectrum (in the paste with vaseline oil): 3410; 1707, 1668 and 1615 cm^{-1}; lit. values[7]: m.p. 202–203°.

Preparation of 11α,16α-*dihydroxyprogesterone* (V). A solution of I (60 mg) in acetone (1·5 ml) was added to each of twenty conical flasks, each containing 200 ml 36-hr culture of *Streptomyces roseochromogenus* ATCC 3347 growing (28°) on a medium composed of 2% glucose, 0·5% NaCl, 0·5% $CaCO_3$, 1% cornsteep liquor in tap water. After 50 hr incubation (28°, rotary agitation 250 cpm) the mycelium was filtered and washed with distilled water. The filtrate and washings were extracted with CH_2Cl_2 (4 × 1·3 l). The combined extracts were concentrated *in vacuo*, the residue (300 ml) dried over $MgSO_4$ and the solvent removed *in vacuo* to dryness. Crystallization of the residue (1·053 g) from ethyl acetate–EtOH gave 294 mg of V, m.p. 211·5–215°, and 39 mg V, m.p. 216–217°. After chromatography on neutral alumina the mother liquor furnished 202 mg chromatographically homogeneous V, m.p. 211·5–215°. Recrystallization of V (m.p. 211·5–215°) from a mixture of acetone–EtOH yielded pure V, m.p. 211–220°, $[\alpha]_D$ +127° (4·8 mg in 0·5 ml $CHCl_3$), λ_{max} = 240 mμ (ε 13850), IR-spectrum (in paste with vaseline oil): 3346, 1705, 1658 and 1610 cm^{-1}. (Found: C, 72·82, H, 8·70. $C_{21}H_{30}O_4$ requires: C 72·80, H 8·73%.) Lit. values[10]: m.p. 213–215°, $[\alpha]_D$ +128° (*c* 1·03, $CHCl_3$), λ_{max} 240 mμ (ε 14400).

Acknowledgments—We thank Professor B. Camerino (Italia) for a sample of 15α-hydroxyprogesterone, Dr. P. Diassi (U.S.A.) for a sample of 15β-hydroxyprogesterone and Dr. O. Hanč (C.S.R.) for a sample of 16α-hydroxyprogesterone.

[9] S. Bernstein, M. Heller and S. M. Stolar, *J. Amer. Chem. Soc.* **76**, 5674 (1954).
[10] J. Fried, D. Perlman, A. F. Langlykke and E. O. Titus, U.S. Pat. 2855343, 1958.