APPENDIX

Nuclear-Magnetic-Resonance and Mass-Spectral Analysis of Ranol Tetra-acetate

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Ranol tetra-acetate has been examined by the nuclear-magnetic-resonance and mass-spectroscopy techniques: the results are compared with those obtained with cyprinol tetra-acetate.

EXPERIMENTAL

Nuclear-magnetic-resonance spectra were recorded on a Varian A-60 spectrometer with deuteriochloroform as solvent and tetramethylsilane as an internal reference. Results are expressed as chemical shifts in τ units and coupling constants, J , as cycles/sec. Accuracies are of the order of $\pm 0.02\tau$ for the chemical shift and ± 0.5 cycle/sec. for J . Mass spectra were recorded on an Atlas mass spectrometer by Dr R. Ryhage of the Karolinska Institute, Stockholm.

RESULTS AND DISCUSSION

(Figures in parentheses are the corresponding values for cyprinol tetra-acetate.)

A striking feature of the nuclear-magnetic-resonance spectrum of ranol tetra-acetate is the very close resemblance of the chemical-shift values to those of cyprinol tetra-acetate (Cross, 1964). Besides the singlet resonances for the C-18-methyl protons at 9.31τ (9.31) and C-19-methyl protons at 9.19τ (9.19), there are no other distinct well-resolved methyl-proton resonances. Thus there is no evidence for a C-27-methyl group. Nine acetate ester protons resonate at 7.96τ (12 acetate protons at 7.94), and a further 3-proton singlet at 7.92τ shows that the fourth acetate has an environment different from those of cyprinol tetra-acetate and is therefore on the side chain, since chemical correlations have established the structure and substitution of the steroid nucleus. Identity of the structure, stereochemistry and substitution pattern of the steroid ring system in cyprinol tetra-acetate and ranol tetra-acetate is strongly suggested by the marked similarities noted above and below.

At lower fields two groups of resonances occur, each equivalent to three protons. Resonance at about 5.0τ (5.0) corresponds to three protons in the environment $\text{CH}\cdot\text{O}\cdot\text{CO}\cdot\text{CH}_3$. The low half-band width, 11 cycles/sec. (13), of these superimposed resonance multiplets indicates that where these

protons are on rings they have an equatorial orientation, thus providing support for the $3\alpha,7\alpha$ -orientation of the acetate groups in a *trans*-A-B-fused steroid ring system. At higher fields, 5.93τ (5.93), a multiplet resonance equivalent to an equatorial (12β) proton on a carbon atom bearing a hydroxyl group is overlaid by a 2-proton multiplet (triplet?) that I attribute to two magnetically equivalent protons immediately adjacent to acetate in the environment $\text{CH}_2\cdot\text{CH}_2\cdot\text{O}\cdot\text{CO}\cdot\text{CH}_3$. Hence there is no extra carbon atom (C-27) substituent at C-25. This is a distinct, and noticeable, difference from the spectrum of cyprinol tetra-acetate which shows a strong doublet (J , 5.5 cycles/sec.) for the four methylene protons of

In agreement with the known chemical evidence, the acetate group in ranol tetra-acetate that is absent from cyprinol tetra-acetate may be placed at C-24, which gives a structure (I; R = H) for ranol, compatible with all known facts, besides being structurally analogous to scymnol. Ranol tetra-acetate then has structure (I; R = $\text{CO}\cdot\text{CH}_3$).

Both cyprinol and ranol have been examined as their tetra-acetates by mass spectroscopy. For both spectra (Fig. 1) the required temperature at the inlet system caused elimination of acetic acid and water from both tetra-acetates so that in neither case was the molecular ion detectable in the spectrum. Instead, each spectrum showed a pattern of prominent m/e peaks, considered to be at $M-78$ and $M-138$, equivalent to the loss of acetic acid plus water and two molecules of acetic acid plus water, respectively, from the molecular ion. In each case much-less-intense m/e peaks appeared at $M-60$ and $M-120$, corresponding to the loss of one or two molecules of acetic acid. This behaviour is typical of bile alcohols (R. Ryhage, personal communication). For ranol tetra-acetate, m/e peaks at $M-198$ (loss of three molecules of acetic acid and one of water) and $M-180$ (loss of three molecules of acetic acid) were also observed.

Comparative study of the mass spectra revealed that for ranol tetra-acetate the series of peaks corresponding to loss of one or two acetic acid

Fig. 1. Mass spectra of (a) cyprinol tetra-acetate and (b) ranol tetra-acetate. Mass spectra are represented only for m/e ratios above 400. Peak intensities are expressed as a percentage of the most intense peak in the spectrum (m/e 253 for both compounds). Peaks with an intensity of less than 1% of the strongest peak are not shown.

(I)

molecules plus water are at m/e ratios consistently lower by 14 mass units than their counterparts in the spectrum of cyprinol tetra-acetate. Therefore, the latter has a molecular formula containing one CH_2 unit more than ranol tetra-acetate. The respective molecular weights are 620 and 606 respectively, based on the $M-138$ and $M-78$ m/e values.

The existence of a similarly substituted steroid tetracyclic nucleus in both ranol and cyprinol was evidenced by intense m/e peaks at 313 and 253 in the mass spectra of both tetra-acetates. These peaks very probably are those for the basic C_{19} tetracycle bearing two double bonds and one acetate (m/e 313),

or three double bonds (m/e 253), which would result from C-17-C-20 bond fission and loss of one or two acetates plus water from the remaining moiety. With a different sampling technique (Atlas Co., Bremen, Germany) the molecular ion peaks were visible at 606 and 620 m/e .

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REFERENCE

Cross, A. D. (1964). *Biochem. J.* **90**, 308.