STRUCTURE and METABOLISM of CORTICOSTEROIDS

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Proceedings of a Symposium Held in Paris 5–6 July 1963 and Organized by the Faculté de Médecine, Laboratoire de Chimie Biologique

Edited by

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PREFACE

This symposium, which was held at the Faculty of Medicine, Paris, in July 1963, is mainly concerned with problems in connection with the chemical structure of corticosteroids. Metabolic transformations of corticosteroids, both *in vitro* and *in vivo*, are also considered.

The main topics dealt with in the reports are: the importance of the magnetic resonance spectra in the study of steroid structure; the relations between the structure of corticosteroids and their form of conjugation in the human organism; and some new pathological aspects of corticosteroid metabolism.

The editors express their gratitude to all the participants of the symposium who have contributed to the preparation of the proceedings for publication.

Paris
February 1964

J. R. Pasqualini
Max F. Jayle

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Pathological Metabolism of Corticosteroids

PART I

Determination of the Structure of Corticosteroids

APPLICATION OF PHYSIOCHEMICAL METHODS IN STRUCTURE DETERMINATION OF STEROIDS*

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Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts, U.S.A.

In the course of studies on the Birch reduction of ring A aromatic steroids and on the ketalization and lead tetra-acetate oxidation of steroids with a dihydroxyacetone moiety, unknown products were formed. The identification of these compounds by a combination of spectroscopic, tracer and chromatographic procedures is discussed. The formation of a 5(10)-monoene on Birch reduction of ring A aromatic steroids was demonstrated. Evidence supporting the presence of a non-conjugated homoannular diene was provided. The identification by n.m.r. spectroscopy of steroidal 20,21-bisethylene dioxides and 17-naphthodioxides was discussed and several generalizations formulated. Formation of 17α-hydroxy-etio-esters and 17α-formyloxy-etio-esters from the oxidation of steroids containing an α-dihydroxyacetone moiety with lead tetra-acetate in alcoholic media was proven. A mechanism for this reaction was proposed, based on tracer studies.

Introduction

Recent developments in physiochemical methods and tracer techniques opened new horizons of scientific endeavour. There is little doubt that the availability of ultra-violet and infra-red spectroscopic instrumentation had a profound influence on the rapid development of the chemistry of natural products in the fifth and sixth decades of this century. The present great breakthrough in several areas of biochemistry probably would not have been possible if not for the advent of isotopic tracers. New tools and probing methods are being continuously added to the scientific arsenal, and the pace seems to be quickening

* This work was supported by grants CY-4663, A-5326 from U.S. Public Health Service and by grants P-102 and P-103 from the American Cancer Society, Inc.

† Recipient of a U.S. Public Health Service Career Program Award CA-K3-16614 from the National Cancer Institute.

almost daily. It is sufficient to glance through recent literature to realize the contribution of new methods, e.g. mass spectroscopy, X-ray diffraction, nuclear magnetic resonance, circular dichroism, optical rotatory dispersion and others, to the development of our knowledge. It would be an impossible task to review even briefly in a single paper the newer methods presently used. However, mention will be made of certain approaches employed in our laboratory which were of great help to us. By necessity, this discussion will be scattered and obviously not exhaustive.

In the course of certain investigations, we had the opportunity to study the Birch reduction[1] of 17β-hydroxy-19-nor-4-methylandrosta-1,3,5(10)-triene.[2] Though elemental analysis, ultra-violet and infra-red spectra of the major product (IIa, m.p. 47–50° C) revealed the reduction of the benzenoid ring, no conclusive structural assignment could be made. Acetylation of (IIa) gave the acetate (IIb, m.p. 101–107°) whose n.m.r. spectrum (Fig. 1) had bands at τ (in $CDCl_3$) 5·18 for the C-17

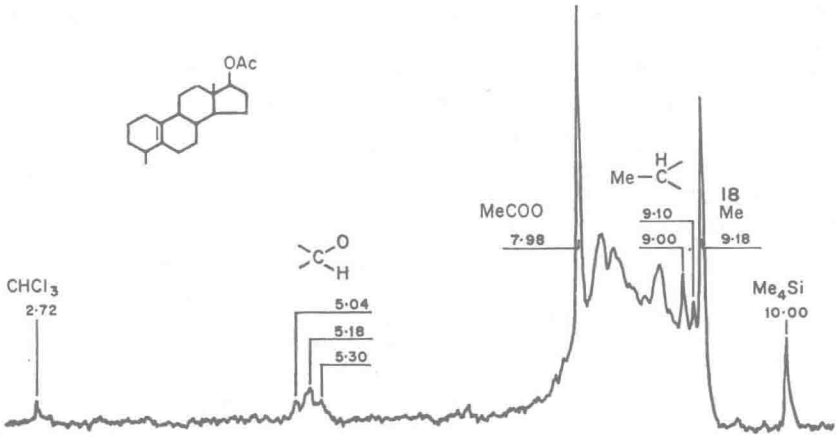
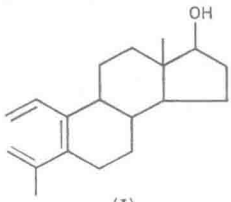


FIG. 1.

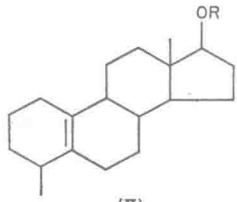
hydrogen, 7·98 for the methyl of the acetate moiety, 9·05 (J = 6·2 c/s) for a secondary methyl, and 9·18 for the C-18 methyl. The spectrum was devoid of bands for vinylic protons. However, when (IIb) was treated with osmium tetroxide, a ditertiary glycol (IIIb, m.p. 204–209°) was formed. The n.m.r. spectrum of (IIIb) had, among others, a signal at τ9·11 (J = 6·0 c/s) for a secondary methyl. Thus it became apparent that the reduction of I led to a fully substituted mono-ene, but it was not certain whether the double bond remained at 5, 10, or isomerized, e.g., to 9,10; 8,9; or 8,14.

Whilst we were investigating the acid catalysed re-arrangement of

17β-hydroxy-17α-methyl products, formation of compounds with a (IVa) structure was observed.[3,4] The chemical shift for the gem-C-17 allylic methyls was about τ9·05. Similarly,[5] in the case of the 17β-methyl compound (IVb), the signal for this methyl was τ9·03 with a coupling constant of J = 6·3 c/s. Analogous observations were reported for other allylic compounds,[6] i.e. for 2,3-seco-1,2-ene-19-methyl, in which the resonance for the 19 methyl was at 8·99. Thus it seems that allylic methyls give signals at about τ9·00–9·05, and that the coupling constant for such secondary methyls is *ab*. J = 6·3 c/s. In the case of (IIb), the observed chemical shift was τ9·05 (J = 6·2 c/s). It appears, therefore, that the C-4 methyl is in an allylic relation to a double bond. Hence, it seems reasonable to assume that the double bond in (II) is between carbons 5 and 10.

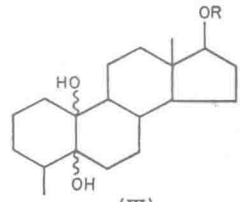


(I)



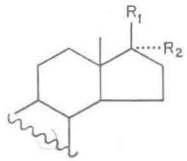
(II)

(a) R = H
(b) R = $COCH_3$



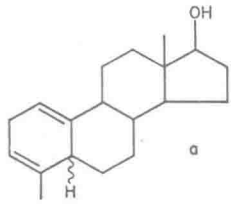
(III)

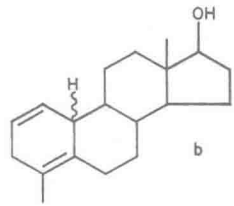
(a) R = H
(b) R = $COCH_3$



(IV)

(a) $R_1 = R_2 = CH_3$
(b) $R_1 = CH_3$; $R_2 = H$





(V)

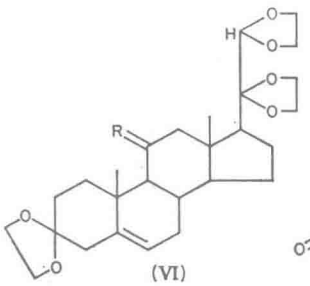
It was rather surprising that reduction of I proceeded to the mono-ene without the formation of a "non-conjugated" homo-annular diene.[1] In order to find out whether a diene or another product was formed in the reaction, we have analysed the crude reduced reaction mixtures by gas–liquid partition chromatography. When the mixture was chromatographed on an F.M. 720 instrument at 250° using a stainless steel column 2 ft × 0·25 in O.D. packed with 20% silicon gum rubber on Chromosorb P (60–80 mesh) with a helium flow rate of 30 ml/min, three peaks were observed. The bands having retention times 430 sec and 600 sec were

identified as I and IIa, respectively. A third band, which was overlapping with the most mobile band, showed a retention time of 480 sec. Though attempts to achieve a better separation were not fruitful, we succeeded in collecting material eluted on the "decreasing" slope of the band having retention time of 480 sec and accumulated enough of the product for an n.m.r. spectrum. The spectrum showed signals at τ (in $CDCl_3$) 4·57 and 6·31 (C-17H), for a methyl group on a double bond τ8·36, and for the C-18 methyl τ9·29. The finding of the resonance for vinylic protons at τ4·57 and of a methyl group on a double bond τ8·36 is consistent with a diene structure (Va). Structure (Vb) is less probable, since this diene would have given a more complex pattern of bands for the vinylic protons. However, other minor signals were also discernible in the spectrum, indicating that the chromatographic eluate was not homogeneous. Till now, attempts to isolate the diene in crystalline form, if indeed present, were not successful. The product proved unstable and apparently re-arranges with ease to the aromatic (I). This was evidenced by gas chromatography, since a decrease of the intensity of the "diene" band occurs with a concomitant increase of intensity of the aromatic band.

Let me quote another, not related example, in which a combination of several methods, but mainly n.m.r. spectroscopy, led to the solution of a problem.[7] In the course of studies on the ketalization of cortisone, in addition to the expected 17β,21-dihydroxypregn-5-en-11-one-3,20-bisethylene-dioxide, two unknown products (VIa) and (VIIa) were isolated. The compounds were correlated by acid hydrolysis of (VIa) to (VIIa). Reduction of (VIa) with lithium aluminium hydride gave (VIb), which could be oxidized to (VIa). Alternately, (VIb) was acid-hydrolysed to (VIIb), and the latter was oxidized to (VIIa). Initially we thought that (VIa) and (VIIa) might be identical to the unknown products reported by Bernstein *et al.*,[8] to which we will assign structures (VIII) and (IX) (see below); but a comparison of their infra-red spectra proved them to be different.

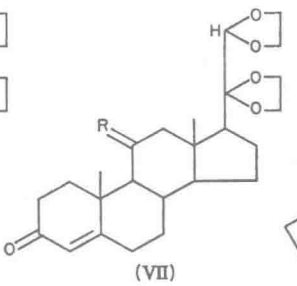
Several routes were considered for the elucidation of structure of the unknown (VI), (VII), (VIII) and (IX). The chemical degradation approach did not appear promising in view of the failure encountered by us and others to remove the protective grouping and to restore a blue tetrazolium-reacting α-ketolic moiety. Inasmuch as ketalization of ketones is acid catalysed, it was considered probable that the dihydroxyacetone moiety of cortisone underwent a Mattox re-arrangement[9] to yield an α-dicarbonyl of the type (X). Our recent observations[10] and observations reported from other laboratories,[11,12] on the ease with which steroids with a dihydroxyacetone chain undergo such re-arrangements in the presence of acids, provided some substance to

this consideration. Since it was reported that α-dicarbonyls on treatment with ethylene-glycol in the presence of acid catalysts yield 2,2′-bisethylene-dioxides and *trans*-1,4;5,8-naphthodioxanes,[13,14] structures of the type (VI) and (VIII) were considered probable.



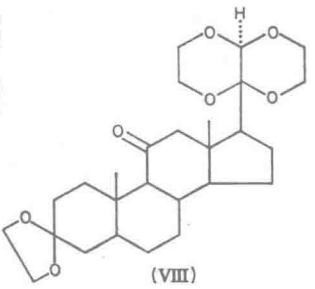
(VI)

(a) R = O
(b) R = OH(β)

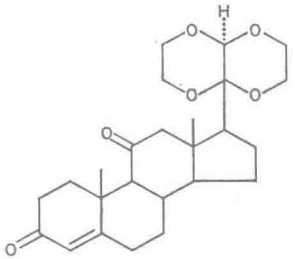


(VII)

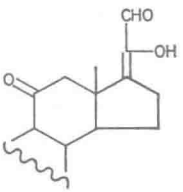
(a) R = O
(b) R = OH(β)



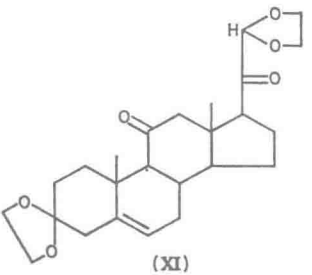
(VIII)



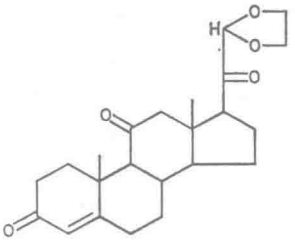
(IX)



(X)



(XI)



(XII)

Having this in mind, we have undertaken an n.m.r. investigation of simple dioxolanes, 2,2′-bisdioxolane and *trans*-1,4;5,8-naphthodioxane with the hope of defining certain spectroscopic correlations enabling the differentiation of the structures.[14] For the interpretation of the

spectra, we have accepted the structural and conformational assignments published in the literature.[13,14] It was found that the single

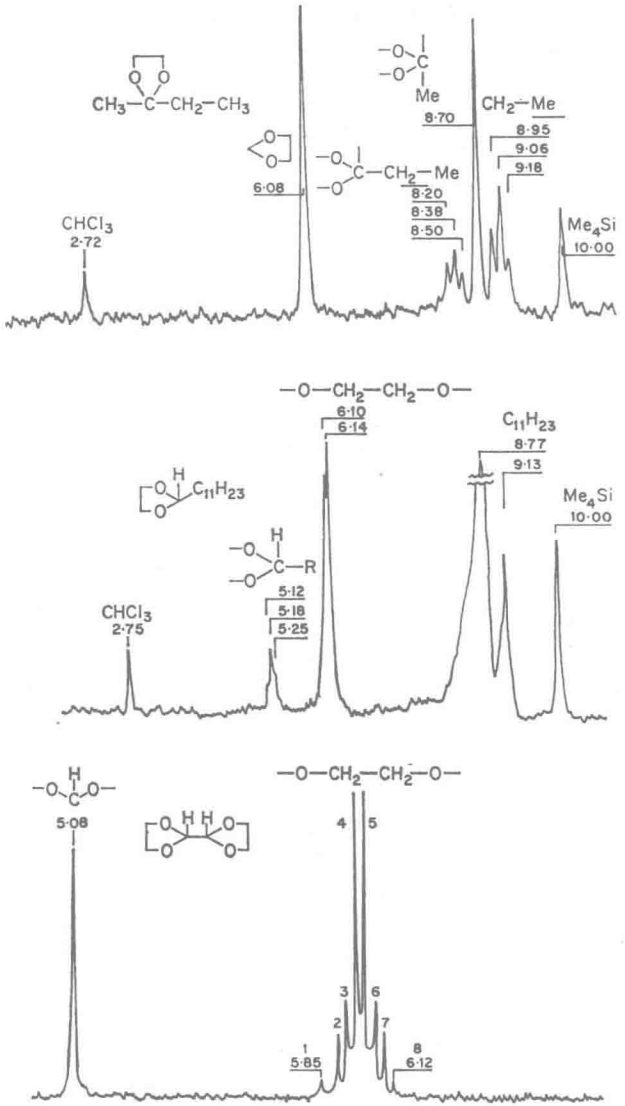


FIG. 2.

proton on a carbon bearing two oxygens in a five-membered ring gives a signal at about τ5·0–5·2 (Fig. 2), whilst a single proton at the bridgehead of *trans*-1,4;5,8-naphthodioxane gives a signal τ5·35 (Fig. 3).

Furthermore, the signal for the $-O-CH_2-CH_2-O-$ moiety of the dioxolane derivative in general did not exhibit resolvable splitting from the coupling between the methylene protons, and hence appeared as a relatively narrow signal. In contrast, the corresponding resonance for naphthodioxane was resolvable into a broad multiplet. These observations and reference values were important features in the subsequent assigning of structures (VI) and (VII). However, since the references

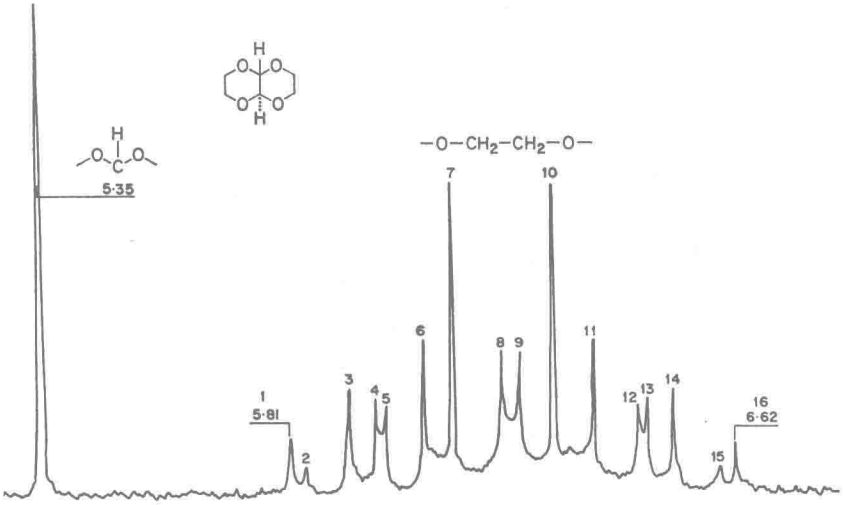


FIG. 3.

for the chemical shifts were obtained for simple dioxolanes and *trans*-1,4;5,8-naphthodioxane, it appeared desirable to verify these for steroids.

Our objective now was to prepare a steroidal aldehydo-ethylene dioxide and to evaluate the chemical shifts for the single proton on the carbon bearing two oxygens and for the $-O-CH_2-CH_2-O-$ group. The route selected for the synthesis was that described by Tsuda *et al.*[15] When cortisone was refluxed with ethylmethyldioxolane in presence of *p*-toluenesulphonic acid, (XI) was obtained. Mild treatment of (XI) with dilute aqueous acetic acid gave (XII). The spectroscopic and analytical results were consistent with the assigned structures (XI) and (XII) (Fig. 4). The n.m.r. spectrum of (XI) had two signals for single protons at τ4·65 and τ5·04. Since C-6 protons on Δ^5 double bonds give signals in the τ4·6–4·8 region, the unsplit resonance at τ5·04 must be assigned to the C-21 proton. In addition, there were two narrow signals, each equivalent to four hydrogens at τ6·00 and τ6·06. The spectrum of (XII) had resonance for single protons at τ4·31 and 5·10 (Fig. 4). The