The Synthesis of Corticosteroids Methylated at C_{21}

For the most part, structural modification of the dihydroxy-acetone side-chain characteristic of anti-inflammatory steroids has been restricted to replacement of one or the other of the critical hydroxyl functions by a hydrogen, halogen, or methyl substituent[1]. Although this approach has produced a number of biologically interesting steroids, none has proved to be useful as an anti-inflammatory agent for systemic application. We have now modified the *intact* side-chain by addition of a methyl group at C_{21}, thereby introducing a critically-placed bulk factor, which also serves to incorporate the C_{21}-hydroxyl function into a new center of asymmetry[2]. This communication describes the synthesis of both C_{21}-epimers of 21-methyl-9α-fluoroprednisolone.

Introduction of the required additional carbon atom was effected directly in a complex starting material by the reaction of prednisolone 21-aldehyde[3] with diazomethane leading to a mixture of products of which the major component was 21-methyl-21, 21a-epoxy-1, 4-pregnadiene-11β, 17α-diol-3, 20-dione (I)[4,5], m. p. 239–241°C, λ_{max} 243 mμ (15,600), $[\alpha]_D$ + 166°. Opening of the oxide ring of I with halogen acids occurred primarily at the terminal atom to afford the desired halohydrins containing a hydroxyl group at C_{21}[6]. These proved to be unusually labile toward a variety of reagents. For instance, bismuth trioxide[7] did not oxidize the chlorohydrin (21-chloromethylprednisolone, II) to the expected 21-chloromethyl-20, 21-diketone, but instead gave the α-diketone, 21-methyl-1, 4-pregnadiene-11β, 17α-diol-3, 20, 21-trione (III)[8], m. p. 211–212°C (dec.), λ_{max} 243 mμ (15,500), $[\alpha]_D$ + 91°, by a novel dehydrohalogenative rearrangement. A similar transformation of the corresponding bromohydrin could be brought about simply by heating in boiling ethyl acetate.

Stereospecific reduction of III to the desired 20, 21-ketol was accomplished in good yield with fermenting yeast[9] affording after acetylation 21-methyl-1, 4-pregnadiene-11β, 17α, 21B-triol-3, 20-dione 21-acetate (IV)[10], m. p. 222–223°C, λ_{max} 244 mμ (14,800), $[\alpha]_D$ + 112°. Application of the familiar sequence for introducing a 9α-fluorine substituent[11] led in four steps to 9α-fluoro-21-methyl-1, 4-pregnadiene-11β, 17α, 21B-triol-3, 20-dione 21-acetate (V), m.p. 251–253°C, λ_{max} 239 mμ (15,350), $[\alpha]_D$ + 87°[12].

An alternative approach was used to prepare the 21-methyl epimer of V. Acidic hydrolysis[13] of 16α, 17α-epoxy-4-pregnene-21-ol-3, 20-dione acetate to the alcohol followed by periodic acid oxidation yielded 16α, 17α-epoxy-3-keto-Δ^4-etiocholenic acid (VI), m. p. 205–207°C, λ_{max} 239 mμ (16,700), $[\alpha]_D$ + 135°. Conversion of VI without isolation of intermediates through the acid chloride, subsequent treatment with *diazoethane*[14], and finally with hydrochloric acid led to 21-methyl-21-chloro-16α, 17α-epoxy-4-pregnene-3, 20-dione (VII), m. p. 200–202°C, λ_{max} 239 mμ (15,700), $[\alpha]_D$ + 124°, *apparently as a single epimer*. The action of potassium acetate in refluxing acetone on VII gave a 2:1 mixture of C_{21}-epimeric acetates from which the predominant 21-methyl-16α, 17α-epoxy-4-pregnene-21A-ol-3, 20-dione 21-acetate (VIII), m. p. 191–194°C, λ_{max} 240 mμ (17,000), $[\alpha]_D$ + 115°, could be separated by chromatography on neutral alumina. Oxide ring opening with hydrobromic acid, Raney nickel debromination[15], microbiological 11β-hydroxylation (*C. lunata*)[16], acetylation, and selenium dioxide dehydrogenation[17] afforded 21-methyl-1, 4-pregnadiene-11β, 17α, 21A-triol-3, 20-dione 21-acetate (IX) (the C_{21}-epimer of IV), m. p. 195–197°C, λ_{max} 243 mμ (13,600), $[\alpha]_D$ + 81°. Application of the standard sequence for 9α-fluorine introduction[11] gave 9α-fluoro-21-methyl-1, 4-pregnadiene-11β, 17α, 21A-triol-3, 20-dione 21-acetate (X) (the C_{21}-epimer of V), m. p. 247–248°C, λ_{max} 239 mμ (14,400), $[\alpha]_D$ + 60°[12].

Pharmacological evaluation[18] reveals V (21-methyl-9α-fluoroprednisolone, B-isomer) to be essentially devoid of electrolyte-regulating activity[19], except for causing slight *natruresis* in adrenalectomized rats. The anti-inflammatory potency of V as measured by the granuloma pouch technique[20] is about six-tenths that of 9α-fluoroprednisolone acetate. Thus, we find that the introduction of a 21-methyl substituent can completely eliminate the powerful sodium retaining properties of the parent 9α-fluoroprednisolone molecule[11,21] while retaining *systemic* anti-inflammatory activity, an effect previously accomplished in 9α-fluoro-C_{21}-oxygenated steroids only with alkyl or hydroxyl substituents at C_{16}. Interestingly, the C_{21}-epimeric compound X shows significantly lower anti-inflammatory activity.

[1] J. FRIED and A. BORMAN, Vitam. u. Horm. *16*, 303 (1958).

[2] The adjacent C_{21}-hydroxyl function is a key determinant of electrolyte-regulating properties. Cf. J. FRIED, Ann. N. Y. Acad. Sci. *61*, 573 (1955).

[3] This compound has been described as a monohydrate (B. G. CHRISTENSEN *et al.*, Chem. & Ind. *1958*, 1259). Our sample (prepared by the same method) was shown by a methoxyl determination to contain one molecule of methanol.

[4] In addition to oxide I at least five by-products were generally formed in yields of less than 5%. One of these was subsequently shown to be the 20,21-diketone III described below.

[5] Satisfactory analytical results were obtained for all new compounds described in this communication. All ultraviolet absorption spectra were taken in 95% ethanol and rotations were determined in dioxane.

[6] Isomeric halohydrins, presumably containing the halogen atom at C_{21}, were invariably formed as minor products in these reactions.

[7] This bismuth-catalyzed rearrangement occurs at a lower temperature (50–60°C) than is usually required to effect acyloin oxidations (W. RIGBY, J. chem. Soc. *1951*, 793), and the formation of elemental bismuth is not observed.

[8] The α-diketone system in III was demonstrated by reaction with *o*-phenylenediamine to form a derivative which exhibited an ultraviolet absorption spectrum typical of quinoxalines, such as that formed from prednisolone 21-aldehyde [W. J. LEANZA *et al.*, J. Amer. chem. Soc. *76*, 1691 (1954)].

[9] C. NEUBERG, *Advances in Carbohydrate Chemistry*, Vol. 4 (Academic Press, Inc., New York, N. Y., 1949), p. 86.

[10] The designation 21B is arbitrarily assigned to the product obtained by the yeast reduction of a 20,21-diketone. The other epimer is designated 21A.

[11] J. FRIED and E. F. SABO, J. Amer. chem. Soc. *79*, 1130 (1957). – R. F. HIRSCHMANN, R. MILLER, J. WOOD, and R. E. JONES, J. Amer. chem. Soc. *78*, 4956 (1956).

[12] Periodic acid cleavage of this compound as the free alcohol afforded 9α-fluoro-3-keto-11β, 17α-dihydroxy-$\Delta^{1,4}$-etiocholadienic acid, identical in all respects with a sample prepared similarly from 9α-fluoroprednisolone.

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[14] A. F. MCKAY *et al.*, Canad. J. Res. *28B*, 683 (1950).

[15] P. L. JULIAN, E. W. MEYER, W. J. KARPEL, and I. R. WALLER, J. Amer. chem. Soc. *72*, 5145 (1950).

[16] G. M. SHULL and D. A. KITA, J. Amer. chem. Soc. *77*, 763 (1955). We are indebted to Mr. J. L. SARDINAS for carrying out this microbiological conversion.

[17] CH. MEYSTRE, H. FREY, W. VOSER, and A. WETTSTEIN, Helv. chim. Acta *39*, 734 (1956).

[18] J. G. LLAURADO and J. A. SCHNEIDER, Fed. Proc. *19*, 159 (1960).

[19] J. G. LLAURADO, Endocrinol. *58*, 390 (1956); Klin. Wschr. *34*, 669 (1956).

[20] The technique employed was essentially that of A. ROBERT and J. E. NEZAMIS, Acta endocrinol. *25*, 105 (1957).

[21] R. O. STAFFORD *et al.*, Proc. Soc. exp. Biol. Med., NY *89*, 371 (1955).

The synthesis of other C_{21}-methyl steroids including the 6α-fluoro analog of V will be described in a subsequent report.

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Zusammenfassung

Die Darstellung der beiden epimeren C_{21}-Methylderivate des 9α-Fluorprednisolons wird beschrieben. 9α-Fluor-21-methyl-1,4-pregnadien-11β,17α,21B-triol-3,20-dion-21-azetat ist ein hochwirksames entzündungshemmendes Steroid, vollkommen frei von Nebenwirkungen der Natrium- und Wasserretention des C_{21}-unsubstituierten 9α-Fluorprednisolons.

Phenol-Mineral Interactions: The Oxidation of Pyrogallol and Other *o*-Diphenols on Silica Gel[1]

Recently, HESS, BACH, and DEUEL discussed evidence for the formation of complexes between *o*-diphenols and various Si-O-Si-compounds, offering them as models for the interactions leading to soil organo-mineral substances[2]. Experimentally, they demonstrated the dissolution of silicagel, alumina, and other minerals in ammoniacal catechol with the formation of crystallizable complexes or compounds. Their highly significant experiments were carried out under comparatively vigorous temperature conditions. It may also be of significance that phenol-mineral interactions take place under mild conditions. Thus, the enzymic oxidation of eugenol to lignin-like polymers is enhanced by highly ordered minerals such as the Amphiboles and Serpentines[3]. Even in the absence of enzyme, chrysotile, hornblende, and muscovite can catalyze (at 25°C) the oxidative polymerization of eugenol to water-insoluble substances which can be eluted from the mineral surface with organic solvents[4].

In the presence of chrysotile, granite or hornblende, the oxidation of catechol at ordinary temperatures led to formation of NaCl-precipitable polymers in 23–32% yield. In their absence, one-tenth as much polymer was formed. The products formed in the presence of different minerals were distinguished by spectroscopic and chemical analysis.

Chrysotile, granite and garnet catalyzed ring hydroxylation and oxidation in phenylalamine at 60°C in the presence of H_2O_2.

In other experiments, glass fibres have been shown to catalyze pyrogallol oxidation in ethanolic solution[5].

Although these systems were set up as models for surface-catalyzed phenol oxidations and differ in approach from the work reported by HESS, BACH, and DEUEL, they may be of value in revealing other aspects of the phenol-mineral interaction.

Experimental. Silicagel[6] was washed three times with hot 10% HCl (1 l/g of gel), soaked in deionized water for 6 h and rinsed in running, de-ionized water for 1 h. The washed product contained no spectrophotometrically detectable impurities, and gave negative tests for Fe (III) and Cu (II).

When M/10 pyrogallol in water or ethanol was poured over a column of washed silicagel, a yellow-brown product formed on the gel surface in less than 1 sec. The product continued to darken on the gel surface and in the supernatant for an additional 10–20 min. The adsorbed pigment was difficultly soluble in alcohol, incompletely soluble in water, but soluble in 10% HCl.

The effect of small quantities of silicagel was followed by photometric determination of oxidation product at 425 mμ. In 50 ml of M/10 KH_2PO_4, 5 *mM* of pyrogallol were dissolved and silicagel added in varying amounts. After they were shaken at 25°C for 1 h, the absorbancies of supernatants and HCl-eluates were determined. The following activity was observed for a 50-mesh gel which had been dried to constant weight over P_2O_5 at 35°C before use:

Silicagel (mg)	Absorbancy at 425 mμ		
	Supernatant	Gel	Total (as%)
0	0·062	—	100
60	0·067	0·019	139
120	0·073	0·021	150
300	0·083	0·056	224
600	0·092	0·085	287

In ethanol, pyrogallol autoxidizes at one-twentieth the rate observed in aqueous media, and the addition of 600 mg silicagel to 5 *mM* of pyrogallol in 50 ml 95% ethanol increased the rate of oxidation 30-fold.

Silica gel is also active in chloroform where pyrogallol autoxidation is nil. Even air-dry silica gel mixed with pyrogallol powder develops a black surface deposit during several weeks at 25°C.

When the gel is heated for 30 min at 500°C, its catalytic activity is lost. Similarly, fine β-quartz powder had no activity at all.

In addition to the commercial product, gels were prepared from sodium metasilicate and tetraethyl orthosilicate $(C_2H_5O)_4$ Si, using HCl or acetic acid. When M/100 HF was used, the gel formed from ethyl orthosilicate was virtually inactive.

The oxidation products formed under aqueous conditions were somewhat variable in composition, but yielded C 48–55%, H 1–2%, and ash 1–2%. These products gave no definite melting point, were lightened somewhat in color by reducing agents, and reacted vigorously with bleaching on addition of peracetic acid. Their ultraviolet absorption characteristics resemble closely those described for catechol polymers formed in the presence of mineral surfaces[4].

Other phenols were also tested as silicagel substrates. Phenol, hydroquinone resorcinol, caffeic acid and chlorophenols were not oxidized, whereas the *o*-diphenols catechol, gallic acid, and dihydroxyphenylalanine (DOPA)

[1] Most of this work was carried out at the University of Rochester with the support of Grant C-2730, National Cancer Institute, U. S. Public Health Service.

[2] R. HESS, R. BACH, and H. DEUEL, Exper. *16*, 38 (1960).

[3] S. SIEGEL, J. Amer. chem. Soc. *79*, 1628 (1957).

[4] S. SIEGEL, Proc. Nat. Acad. Sci. U. S. *43*, 811 (1957).

[5] S. SIEGEL, F. PORTO, and P. FROST, Arch. Biochem. Biophys. *82*, 330 (1959).

[6] Fisher Scientific Co. Silica gels, up to 200 mesh (gas chromatography grade). The ethyl silicate used was a Fisher purified grade, and sodium metasilicate was a certified reagent grade.

[7] The work of ZIECHMAN and KROLL cited in [2]. – F. SCHEFFER and W. KROLL, Agrochimica *4*, 97 (1960).