18-Substituted Steroids. Part 12.[1] Synthesis of Aldosterone 21-Sulphate, and an Improved General Procedure for Preparing Steroid Sulphates

David N. Kirk* and Maruthiandan S. Rajagopalan
Medical Research Council Steroid Reference Collection, Department of Chemistry, Queen Mary College, Mile End Road, London E1 4NS

Treatment of aldosterone with triethylamine–sulphur trioxide in pyridine followed by a convenient purification procedure involved absorption on a C_{18} 'Sep-pak' was found to give aldosterone 21-sulphate. The product was characterised by its 400 MHz ^{1}H n.m.r. spectrum, which showed the presence of four isomeric forms. The improved sulphation and purification procedure has been extended to the preparation of some other steroid sulphates.

The sulphation of steroid hormones and their metabolites by sulphokinases in the liver, testis, placenta, and adrenal cortex was regarded originally as merely a water-solubilising process permitting their excretion through the kidney. Since the early 1960s, however, it has been recognised that the steroid sulphates may themselves be directly involved in biosynthetic pathways.[2] Speculation about the possible physiological role of steroid sulphates has led to the view that they may provide a circulating pool of steroid in the plasma, able to cross cell membranes to reach the site of biological activity, and there be desulphated or metabolised to the active hormone.[3] The conversion of 3β-hydroxyandrost-5-en-17-one 3-sulphate (dehydroepiandrosterone sulphate) into oestrogens in the foetus and placenta is well established.[3,4]

The mineralocorticoid aldosterone is metabolised to a variety of products prior to excretion[5] one of which, the 21-sulphate, has been shown by recent studies[6] to be produced *in vivo* by adrenal glands and *in vitro* by adrenal tissue in cases of human adrenal carcinoma. The sulphate is probably produced also in the normal adrenal glands, though in smaller quantities. To assist in this study and to supply material for evaluation of the mineralocorticoid and any other biological activity, we undertook the chemical synthesis and characterisation of aldosterone sulphate, which has been formerly available in very small quantities as a product of incubation of aldosterone with homogenates of rat liver.[6] The toad bladder has been found to convert aldosterone to a monosulphate, but the point of conjugation is uncertain.[7] Although a chemical synthesis of the 21-monosulphate had been reported[8] following Mumma's method (mediated by dicyclohexylcarbodi-imide),[9] an attempt[10] to repeat the published procedure was frustrated by difficulties in purifying the product.

We now report a chemical synthesis of aldosterone 21-sulphate involving an improved method of isolation which can be adapted to the preparation of steroid sulphates in general. Numerous chemical methods are available for the sulphation of steroidal alcohols.[11,12] Sulphuric acid itself has been used[13] as the sulphating agent in the presence of dicyclohexylcarbodi-imide (DCC), with dimethylformamide (DMF) as solvent. This offers a method for selective sulphation at unhindered sites of polyhydroxy steroids, but the use of sulphur trioxide complexes[14] is generally preferred, particularly for acid-sensitive steroids. Pyridine–sulphur trioxide[15] has often been the reagent of choice[16] though more recently trimethylamine–sulphur trioxide[17] and triethylamine–sulphur trioxide[18] have been recommended owing to their higher solubilities in pyridine, the commonly used solvent.

A preliminary trial of the partial sulphation of aldosterone with an equimolar amount of triethylamine–sulphur trioxide in pyridine at room temperature resulted in only slight conversion, determined by thin layer chromatography (t.l.c.), but the presence of only a single component more polar than the starting material was encouraging. Experiments aimed at optimising the reaction conditions for selective sulphation at C-21 revealed that it was best to employ a 1.4 molar excess of the reagent and a reaction period of four hours at room temperature (20 °C). Prolonged reaction times or the use of a larger excess of the reagent led to over-sulphation, as observed by the presence of compounds less retained than the monosulphate and the starting material on reverse phase high performance liquid chromatography (h.p.l.c.) on a C_{18} μ-Bondapak column.

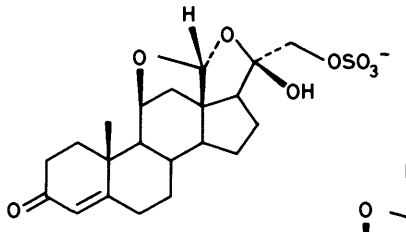
It is convenient[19] in partial sulphations to separate steroid sulphates from their parent alcohols by quenching the reaction mixture in an aqueous medium, followed by initial extraction with a relatively non-polar solvent such as diethyl ether to remove the free steroid. Further extraction with a highly polar solvent such as butan-1-ol then affords the sulphates. Accordingly, we developed a procedure involving an aqueous work-up, bearing in mind the need to maintain the pH as close to neutrality as possible owing to the sensitive nature of aldosterone to acidic† or alkaline‡ conditions. Dichloromethane was found to be more suitable than diethyl ether for the recovery of unchanged aldosterone, while an aqueous medium containing ammonium acetate ensured an essentially neutral pH. However, extraction of the sulphate with butan-1-ol, though quantitative, caused problems associated with the low volatility of the solvent, often resulting in gum-like products from which it was not possible to crystallise aldosterone 21-sulphate. This difficulty was easily overcome by the application of the C_{18} 'Sep-pak' extraction procedure recently introduced into biochemical methodology.[20,21] When the aqueous solution containing aldosterone sulphate was passed through a C_{18} 'Sep-pak', the sulphate was completely retained. After washing the 'Sep-pak' with water to remove inorganic salts, aldosterone sulphate (as ammonium salt) was quantitatively eluted with methanol, and was easily purified by crystallisation.

The structure of aldosterone 21-sulphate was confirmed from its elemental analysis, which clearly indicated monosulphation, from the recovery of aldosterone by the action of sulphatase,[6] and from its particularly interesting n.m.r. spectral characteristics.

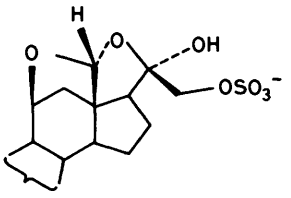
Proton n.m.r. evidence[26,27] has indicated that free aldosterone and related compounds exist in solution as

† Under acidic conditions aldosterone undergoes intramolecular dehydration to the 11,18:18,21-doubly bridged ether,[22] which has been used as a derivative for g.l.c. analysis.[23]

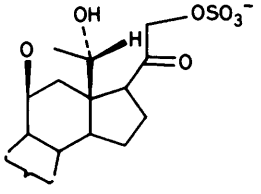
‡ In the presence of alkali, aldosterone rearranges to a variety of products depending upon the experimental conditions.[24,25]



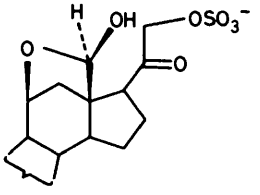
(1a)
(18*R*),(20*S*)



(1b)
(18*R*),(20*R*)



(2a)
(18S)



(2b)
(18R)

equilibrium mixtures of the doubly cyclised 18,20-hemiacetal form and the open-chain 11,18-hemiacetal-20-oxo form. Crystalline aldosterone (monohydrate) was found from *X*-ray crystallographic analysis to be solely in its (20*S*)18,20-hemiacetal form.[28] The 100 MHz spectrum of the 21-monosulphate showed it to be a mixture, but was insufficiently resolved to permit an unambiguous analysis. The 400 MHz spectrum, however, gave clear indications of the presence of *four* isomers, a pair of epimers (**1a**) and (**1b**) at C-20 of the 18,20-hemiacetal form, and a pair of epimers (**2a**) and (**2b**) at C-18 of the 11,18-hemiacetal-20-oxo form. The location of the sulphate group in all four isomers at C-21, rather than at C-18, was evident from additional downfield shifts of the 21-H signals by *ca.* 0.8 p.p.m., compared with corresponding signals in the spectrum of aldosterone itself, whereas the chemical shifts of the 18-H signals were not significantly different from those observed for free aldosterone in its two major tautomeric forms. Most of the signals in the low-field part of the spectrum (*ca.* δ 2.5—6) could be assigned either to the major of the two 18,20-hemiacetal forms (**1a**), which was the predominant component of the four, or to the major of the two 11β,18-hemiacetal-20-oxo forms (**2a**), from consideration of their chemical shifts, relative intensities and coupling constants. Some assignments were confirmed by spin-decoupling experiments. The conclusions are presented in the Table, with corresponding data for aldosterone itself, where available. Signals due to 21-H_2 in the two 20-oxo forms (**2a**) and (**2b**) appeared as a strong and a weak double doublet (AB system), characterised by large geminal coupling constants (J_{gem} 15—17 Hz), whereas 21-H_2 in the 18,20-hemiacetals gave narrower double doublets (J_{gem} *ca.* 10 Hz) at somewhat higher field; one of these signals, assigned to the major 18,20-hemiacetal form (**1a**), was barely resolved in the 400 MHz spectrum, appearing virtually as a singlet at low scale expansion. The best resolved signals in the spectrum were the four distinct doublets for 11α-H. Assignments of these signals to the respective isomers (Table) were based on comparisons of their integrated intensities with those of the more distinctive 21-H_2 signals, and indicated that the 18,20-hemiacetal forms are those which shift the 11α-H signal farthest to low-field. The separate integrals of the four 11α-H signals indicated the composition of the mixture of isomeric aldosterone sulphates in solution (in C_5D_5N at ambient temperature) as *ca.* 63% (**1a**): 8% (**1b**): 24% (**2a**): 5% (**2b**). Distinctions between the epimeric 18,20-hemiacetals (**1a**) and (**1b**) are made on the basis that the (18*R*,20*S*) epimer (**1a**) is sterically the less strained and less crowded, and similarly the (18*S*) 11β,18-hemiacetal 20-oxo (**2a**) is less crowded than its (18*R*) epimer (**2b**) (Dreiding models). Another point of interest in the tabulated n.m.r. data for aldosterone sulphate is the 12β-H signal. Two double doublets appear in the region 2.7—3.3, each arising from a combination of geminal (12α,12β) and vicinal (11α,12β) couplings. The

Table. Main features of the ^{1}H-n.m.r. spectrum of aldosterone 21-ammonium sulphate (at 400 MHz in C_5D_5N). Corresponding data for free aldosterone, where available, are included in square brackets for comparison

Structure	19-H_3	12β-H	21-H_2	11α-H	18-H	4-H
Major 18,20-hemiacetal (**1a**) (18*R*,20*S*)	1.215 (s)	2.735 (dd, J_{gem} 11 Hz, J_{vic} 6 Hz)	4.795, 4.805 (dd, J_{gem} 10 Hz)	4.65 (d, *J* 6 Hz)	5.80 (s)	5.825 (br s)
	[1.205 (s)]	[2.735 (dd, J_{gem} 10 Hz, J_{vic} 6 Hz]	[3.99, 4.09 (dd of ds* J_{gem} 12 Hz)]	[4.80 (d, *J* 6 Hz)]	[5.875 (s)]	[5.845 (br s)]
Minor 18,20-hemiacetal (**1b**) (18*R*,20*R*)			4.83, 4.93 (dd, J_{gem} 10 Hz)	4.60 (d, *J* 6 Hz) [4.84 (d, *J* 6 Hz)]		
Major 20-oxo form (**2a**) (18*S*)	1.23 (s)	3.30 (dd, J_{gem} 10.5 Hz J_{vic} 6.5 Hz)	5.43, 5.51 (dd, J_{gem} 15 Hz)	4.46 (d, *J* 6 Hz)	5.35 (s)	5.84 (br s)
	[1.23 (s)]	[*ca.* 3.03 (dd?)]	[4.69, 4.985 (dd of ds* J_{gem} 16 Hz)]	[4.59 (d, *J* 6 Hz)]	[5.385 (d, $J_{18.OH}$ 5 Hz)]	[5.86 (br s)]
Minor 20-oxo form (**2b**) (18*R*)			5.225, 5.295 (dd, J_{gem} 17 Hz)	4.405 (d, *J* 6 Hz)		

* $J_{21A.OH}$ 5 Hz, $J_{21B.OH}$ 6 Hz.

individual assignments to (**1a**) and (**2a**) indicated by the signal integrals were confirmed by selective spin-decoupling from 11α-H in the respective isomers.

We have successfully extended the C_{18} 'Sep-pak' method of purification to the preparation of the 21-monosulphates of cortisone, corticosterone, cortisol and 3β,21-dihydroxypregn-5-en-20-one, with only minor variations during work-up (see Experimental section). We also used this method to purify androst-5-ene-3β,17β-diol 3-monosulphate obtained by the reduction of 3β-hydroxyandrost-5-en-17-one 3-sulphate with sodium borohydride. We recommend this as a generally advantageous procedure.

For relatively large scale syntheses, where a single C_{18} 'Sep-pak' cartridge is unsuitable owing to its very limited loading capacity (*ca.* 10 mg), the same effect can be achieved after extraction of any unchanged starting material, by pumping aqueous steroid sulphate through a conventional h.p.l.c. system equipped with a C_{18}-reverse phase bonded packing, washing the system with water until free of inorganic salts, and finally pumping methanol through the column to elute the conjugate. It is also possible to carry out ion exchange on the reverse phase column after retention of the conjugate by washing, with the appropriate cationic acetate in aqueous solution, prior to elution with methanol.

Experimental

M.p.s were determined on a Reichert melting microscope. I.r. spectra were determined for potassium bromide discs. ^{1}H N.m.r. spectra were determined by Dr. R. D. Farrant on the University of London Intercollegiate Research Service (ULIRS) Bruker WH-400 at Queen Mary College, at 400 MHz using tetramethylsilane as an internal standard.

Water was distilled from glass and was freed from any organic contaminants by passage through a 'Norganic' cartridge (Millipore Corporation, Bedford, Massachusetts 017300). All solvents were purified before use.[29] Triethylamine–sulphur trioxide was freshly prepared by the method of Tsreng and Klein.[30] C_{18}-'Sep-pak' cartridges (Waters Associates) were primed with methanol and washed with water before use.

Aldosterone 21-Ammonium Sulphate.—Triethylamine–sulphur trioxide (20 mg, 0.11 mmol) was added to a solution of aldosterone (25 mg, 0.07 mmol) in dry pyridine (0.05 ml). The mixture was left for 4 h at 20 °C, when an aqueous solution of ammonium acetate (2.0 ml, 5%) was added and unchanged aldosterone was extracted with dichloromethane (3 × 2.0 ml).

The organic layers were bulked and washed with aqueous ammonium acetate (2 × 1.0 ml); aldosterone (7 mg) was recovered by evaporation of the solvent. The aqueous extracts containing the conjugate were pooled and subjected briefly to reduced pressure at room temperature on a rotary evaporator to remove traces of dichloromethane. The solution was then passed through a C_{18}-'Sep-pak'. The 'Sep-pak' was washed successively with aqueous 5% ammonium acetate (2.0 ml) and water (5 ml) and these washings were rejected. Elution with methanol (2.5 ml) followed by evaporation of the solvent furnished a gum from which aldosterone 21-monosulphate (8 mg) crystallised as its ammonium salt upon addition of acetone (a few drops): m.p. 176—178 °C (lit.,[8] 135—138 °C); $\nu_{max.}$ 1 660, 1 607, 1 440, 1 410, and 1 230 cm^{-1} (Found: C, 51.8; H, 6.8; N, 2.9; S, 6.6. $C_{21}H_{31}O_8NS\cdot1.5H_2O$ requires C, 52.1; H, 7.0; N, 2.9; S, 6.6%).

The identity of this product compared with aldosterone 21-sulphate obtained from rat liver homogenate has been confirmed by its chromatographic behaviour, negative reaction with alkaline blue tetrazolium reagent, positive reaction with methylene blue, and cleavage by a sulphatase enzyme to give aldosterone.[10]

Corticosterone 21-Monosulphate.—Corticosterone (100 mg, 0.289 mmol) in dry pyridine (0.3 ml) was treated with triethylamine–sulphur trioxide (60 mg, 0.33 mmol) for 45 min at 20 °C. Aqueous 5% potassium acetate (20 ml) was added and the clear solution was extracted with ether (4 × 15 ml). The ether extracts were washed with water (2 × 3 ml), the washings combined with the main bulk of the aqueous steroid sulphate solution and subsequently left on a rotary evaporator at room temperature to remove traces of ether. After filtration the aqueous phase was passed at the rate of 4 ml per min through a conventional h.p.l.c. system equipped with a stainless steel column (length 25 cm; internal diameter 1 cm) packed with Spherisorb ODS, primed with methanol and equilibrated in water before use. This was followed by pumping through successively with aqueous 5% potassium acetate (20 ml) and water (50 ml). The corticosterone sulphate was retained on the column as the potassium salt. The washings did not exhibit any absorption at 238 nm. Methanol (50 ml) quantitatively eluted the conjugate, which was isolated by evaporation of the solvent under reduced pressure at 40 °C, followed by crystallisation of the residue (130 mg) from methanol containing 0.1% potassium acetate. The product (105 mg), m.p. 174—177 °C (lit.,[31] 178—180 °C) was chromatographically homogeneous, $\nu_{max.}$ 3 430, 1 705, 1 660, 1 607, and 1 220—1 280 cm^{-1}.

Cortisol 21-Monosulphate and Cortisone 21-Monosulphate.—The experimental conditions employed to prepare these sulphates were as described above for corticosterone sulphate, except that improvisation was found necessary during isolation of cortisone 21-monosulphate, which crystallised partially from the aqueous phase during the second extraction with ether. These crystals were filtered, redissolved in sufficient water and combined with the rest of the aqueous solution prior to pumping into the h.p.l.c. system. Dilution (total volume > 100 ml) did not prevent retention of the sulphate on the reverse-phase column. Cortisone sulphate, isolated as the ammonium salt, crystallised from methanol containing 0.1% ammonium acetate; m.p. 280—285 °C (lit.,[18] 275 °C); $\nu_{max.}$ 3 400, 3 200—3 220, 1 732, 1 700, 1 650, 1 430, and 1 205—1 240 cm^{-1}. Cortisol sulphate, isolated as the potassium salt, crystallised from methanolic potassium acetate (0.1%); m.p. 175—178 °C (lit.,[32] 179 °C); $\nu_{max.}$ 3 400—3 460, 1 715, 1 650, and 1 230—1 280 cm^{-1}.

3β,21-Dihydroxypregn-5-en-20-one 21-Potassium Sulphate.—3β,21-Dihydroxypregn-5-en-20-one 3-acetate[33] (100 mg) was sulphated essentially as above to the pyridinium salt. This was subjected to alkaline hydrolysis of the 3-acetate by boiling for 45 min in aqueous 50% methanol (6 ml) containing potassium hydroxide (1.0 mmol) (*cf.* preparation of pregnanediol 3-monosulphate[19]). The solution was cooled to room temperature, and aqueous 10% acetic acid was added to bring the pH to *ca.* 6.5. After evaporation of the methanol on a rotary evaporator at 40 °C, sufficient water (25 ml) was added to keep the conjugate in solution. The sulphate was then isolated as the potassium salt by the h.p.l.c. technique described above. Crystallisation from methanol containing 0.1% potassium acetate furnished the *title compound* (90 mg), m.p. 215—219 °C; $\nu_{max.}$ 3 400—3 460, 1 718, and 1 220—1 300 cm^{-1}; δ(CD_3SOCD_3) 0.54 (s, 18-H_3), 0.94 (s, 19-H_3), 3.0—3.6 (m, 3-H_1 superimposed on the water peak), 4.25 (s, 21-H_2) and 5.18 (m, 6-H_1) (Found: C, 52.4; H, 6.9; S, 7.0. $C_{21}H_{31}O_6SK\cdot1.5H_2O$ requires C, 52.8; H, 7.2; S, 6.7%).

Androst-5-ene-3β,17β-diol 3-Ammonium Sulphate.—3β-Hydroxyandrost-5-en-17-one (100 mg) was sulphated to give the pyridinium salt by the method described[34] for the sulphation of 17β-hydroxy-5α-androstan-3-one. The crude 3-sulphate (145 mg) was dissolved in water (30 ml) and treated

with sodium borohydride (50 mg) for 30 min at room temperature, when aqueous acetic acid (2.0 ml, 6%) followed by sodium acetate (300 mg) were added. After filtration the solution was pumped through the reverse-phase column as above. The column was washed successively with ammonium acetate (50 ml, 2%), water (20 ml) and methanol (20 ml). Evaporation of the methanolic eluate, followed by crystallisation of the residue from methanol containing 0.1% ammonium acetate, furnished the 3-monosulphate as the ammonium salt (108 mg), m.p. 208—210 °C (lit.,[3] m.p. 206—209 °C); $\nu_{max.}$ 1 220—1 270 (sulphate) and 1 400 cm^{-1} (ammonium ion). Solvolysis by the method of Burnstein and Liebermann[35] furnished androst-5-ene-3β,17β-diol as the sole product.

Use of C_{18}*-Bonded Silica as an Ion-Exchange Column.*—3β-Acetoxy-21-hydroxypregn-5-en-20-one[33] (50 mg) was sulphated[34] to give the pyridinium salt and the crude product (70 mg) was dissolved in aqueous triethylamine (20 ml, 5%). The pH was lowered to 6.5 by the addition of aqueous acetic acid (2%) and the solution was pumped through the h.p.l.c. system as above. This was followed successively with aqueous triethylamine–acetic acid (20 ml; pH 6.5) and water (20 ml), then the conjugate was eluted with methanol (20 ml). Evaporation of the methanolic eluate furnished the 21-sulphate as its triethylammonium salt (80 mg), free from any detectable pyridinium salt; δ(CD_3SOCD_3) 0.55 (s, 18-H_3), 0.98 (s, 19-H_3), 1.2 (t, methyl protons of the triethylamine moiety), 2.00 (s, 3-OCOMe), 3.04—3.16 (q, *J* 8 Hz, methylene protons of the triethylamine moiety superimposed on the solvent peak), 4.25 (s, 21-H_2), *ca.* 4.4 (m, 3-H_1), and 5.28 (m, 6-H_1). There were no signals in the aromatic region, indicating the absence of the pyridinium ion.

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References

1 Part 11, R. Das and D. N. Kirk, *J. Chem. Soc., Perkin Trans. 1*, 1984, 1821.
2 H. I. Calvin, R. VandeWiele, and S. Liebermann, *Biochemistry*, 1963, **2**, 648.
3 H. I. Calvin and S. Liebermann, *J. Clin. Endocrinol. Metab.*, 1966, **26**, 402.
4 D. A. Kelly and J. K. Grant, *Eur. J. Steroids*, 1967, **2**, 209.
5 D. J. Morris, *Endocr. Rev.*, 1981, **2**, 234.
6 T. P. Jowett and J. D. H. Slater, *J. Steroid Biochem.*, 1983, **18**, 471.
7 D. J. Morris, M. M. McDermott, S. A. Latif, A. Keating, and C. J. Kenyon, *J. Steroid Biochem.*, 1981, **15**, 473.
8 J. H. Grose, W. Nowaczynski, O. Kuchel, and J. Genst, *J. Steroid Biochem.*, 1973, **4**, 551.
9 R. O. Mumma, C. P. Hoiberg, and W. W. Weber, *Steroids*, 1969, **14**, 67.
10 Dr. J. D. H. Slater, personal communication.
11 H. E. Hadd and P. Blickenstaff, 'Conjugates of Steroid Hormones,' Academic Press, New York, 1969.
12 S. Bernstein and S. Solomon, 'Chemical and Biological Aspects of Steroid Conjugation,' Springer Verlag, New York, 1970.
13 R. O. Mumma, *Lipids*, 1966, **1**, 221; C. P. Hoiberg and R. O. Mumma, *J. Am. Chem. Soc.*, 1969, **91**, 4273.
14 E. E. Gilbert, *Chem. Rev.*, 1963, **62**, 549.
15 H. H. Sisler and L. F. Audrieth, *Inorg. Synth.*, 1946, **2**, 173.
16 A. E. Sobel and P. E. Spoerri, *J. Am. Chem. Soc.*, 1941, **63**, 1259.
17 H. Fex, K. E. Lundwall, and A. Ollson, *Acta Chem. Scand.*, 1968, **22**, 254.
18 J. P. Dusza, J. P. Joseph, and S. Bernstein, *Steroids*, 1968, **12**, 49.
19 M. S. Rajagopalan and A. B. Turner, *J. Chem. Soc. C*, 1969, 1858.
20 J. Goto, H. Kato, Y. Saruta, and T. Nambara, *J. Liq. Chromatogr.*, 1980, **3**, 991; J. O. Whitney and M. M. Thaler, *ibid.*, 1980, **3**, 545; C. H. L. Shackleton and J. O. Whitney, *Clin. Chim. Acta*, 1980, **107**, 231.
21 N. Murata, T. Beppu, H. Takikawa, H. Otsuka, T. Kasama, and Y. Seyama, *Steroids*, 1984, **42**, 575.
22 S. A. Simpson, J. F. Tait, A. Wettstein, R. Neher, J. von Euw, O. Schindler, and T. Reichstein, *Helv. Chim. Acta*, 1954, **37**, 1163.
23 C. J. W. Brooks, *Proc. Ass. Clin. Biochem.*, 1963, **2**, 53; S. J. Gaskell and C. J. W. Brooks, *J. Chromatogr.*, 1978, **158**, 331.
24 D. N. Kirk and B. W. Miller, *J. Steroid Biochem.*, 1982, **16**, 269.
25 V. R. M. Mattox, P. C. Carpenter, and E. Graf, *J. Steroid Biochem.*, 1981, **14**, 19.
26 P. Genard, 'Contribution à la determination et de la configuration et de la conformation molecularies des corticosteroides,' Masson, Paris, 1974.
27 D. N. Kirk and B. W. Miller, *J. Chem. Soc., Perkin Trans. 1*, 1980, 2818.
28 'Atlas of Steroid Structure,' ed. W. L. Duax and D. A. Norton, IFI/Plenum, London, Vol. 1, 1975.
29 D. D. Perrin, W. F. Armarego, and D. D. Perrin, 'Purification of Laboratory Chemicals,' Pergamon, London, 1966.
30 K. Y. Tsreng and P. D. Klein, *J. Lipid Res.*, 1977, **18**, 491.
31 J. A. Pasqualini, R. Zelnik, and M. F. Jayle, *Bull. Soc. Chim. Fr.*, 1962, 1171.
32 E. Griebsch and W. Garn, U.S.P. 3 152 044, Oct. 6, 1964.
33 F. Giral, *J. Am. Chem. Soc.*, 1950, **72**, 1913.
34 D. Baxendale, D. N. Kirk, M. S. Rajagopalan, and A. B. Turner, *J. Chem. Soc. C*, 1971, 2563.
35 S. Bernstein and S. Liebermann, *J. Biol. Chem.*, 1958, **233**, 331.

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