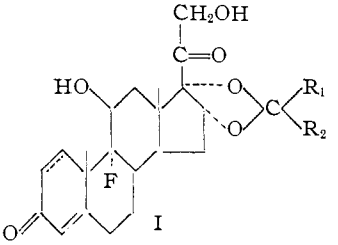
TABLE I

Derivative of	with	M.p., °C.	$[\alpha]_D^{CHCl_3}$	Analyses, found, % C	H	Liver glycogen[a] Cortisone acetate = 1	Anti-inflammatory[b] Cortisol acetate = 1
Δ^1-FF-16α-ol[c]	Acetaldehyde (I)	244–246	+102°	65.47	7.19	98 (62–155)[d]	50
FF-16α-ol	Acetaldehyde (II)	244–247	+145°	65.34	7.48	94 (57–157)	30
Δ^1-FF-16α-ol	Acetone (III)	292–294	+109°	66.49	7.31	92 (48–176)	40
FF-16α-ol	Acetone (IV)	270–273	+137°	66.03	7.92	121 (47–314)	18
Δ^1-FF-16α-ol	Methylethyl ketone (V)	255–260	+ 92°	67.01	7.41	77 (49–121)	35
Δ^1-FF-16α-ol	Diethyl ketone (VI)	265–268	+ 91°	67.62	7.24	26 (16–43)	11
Δ^1-FF-16α-ol	Methylisobutyl ketone[e] (VII)	256–258	+ 89°	68.10	7.72	12 (8–18)	8
Δ^1-FF-16α-ol	Methylisobutyl ketone (VIII)	185–188	+ 88°	67.87	7.74	4 (2–7)	3
Δ^1-FF-16α-ol	Cyclohexanone (IX)	278–281	+ 90°	67.97	7.53	16 (10–25)	5
Δ^1-FF-16α-ol	Acetophenone[f] (X)	281–283	+ 23°	69.91	7.04	12 (7–21)	15
Δ^1-6α-methyl-FF[g] (XI)						60 (34–106)	22

[a] Modifications of assay described by Pabst, *et al.*, *Endocrinology*, **41**, 55 (1947). [b] According to F. M. Singer and A. Borman, *Proc. Soc. Exptl. Biol. Med.*, **92**, 23 (1956). [c] FF = 9α-fluorocortisol. [d] The figures in parentheses represent the 95% confidence limits. [e] The two sets of values refer to the two stereoisomers about the new asymmetric center. [f] Shows infrared bands at 13.06 and 14.29 μ characteristic of mono-substituted phenyl. [g] We wish to express our sincere thanks to Dr. G. Schreiber of the Upjohn Company for supplying this sample.

a trace of a mineral acid, preferably perchloric acid, until solution has occurred. Characterizing data and biological properties for some representative derivatives are shown in Table I.[4] They reduce tetrazolium reagent and form monoacetates *e.g.*, 9α-fluoro-16α-hydroxyprednisolone acetonide 21-acetate (m.p. 266°; $[\alpha]^{23}D$ +92° (*c* 0.59 in $CHCl_3$); λ_{max}^{Nujol} 3.01, 5.71, 5.79, 6.01–6.04, 6.21–6.24 μ. *Anal.* Found: C, 65.49; H, 6.81), and are therefore formulated as 16α,17α-ketals or acetals of structure I. In contrast to the extreme



I

ease of hydrolysis of most cyclic ketals the acetonide of 9α-fluoro-16α-hydroxyprednisolone remains unchanged during 4 hours of refluxing with 0.1 *N* sulfuric acid in aqueous methanol. The biological data indicate a progressive increase in activity with decreasing molecular weight of R_1 and R_2, with the exception of R_1 = phenyl. The majority of the derivatives listed show considerably greater glucocorticoid and anti-inflammatory activity than the parent steroids,[5] the more active ones surpassing 6α-methyl-9α-fluoroprednisolone[6] the most potent glucocorticoid heretofore described. The anti-inflammatory glucocorticoid activity ratios are in each case greater than those found for the respective parent compounds. Compounds I, II, III, IV and V cause sodium excretion in the rat, IX and X cause retention, and VI and XI effect neither retention nor excretion. In view of the altered physiological properties and of the unusual acid stability this group of compounds, in our opinion, is biologically active *per se* rather than after hydrolysis to the parent compounds.

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(4) All infrared spectra (Nujol) show bands characteristic of 20-keto (5.80–5.85 μ), Δ^4-3-keto (6.01 and 6.15 μ) and $\Delta^{1,4}$-3-keto groups (5.99–6.02, 6.15–6.19 and 6.21–6.24 μ), respectively.

(5) The liver glycogen and anti-inflammatory values determined in our laboratories are: 9α-fluoro-16α-hydroxyprednisolone, 14 (9–22) and 4; 9α-fluoro-16α-hydroxycortisol, 11 (7–19) and 1.

(6) G. B. Spero, J. L. Thompson, F. H. Lincoln, W. P. Schneider and J. A. Hogg, THIS JOURNAL, **79**, 1515 (1957).

THE SYNTHESIS OF DIHYDROSPHINGOMYELIN

Sir:

The correctness of structure I for the sphingomyelins has been proved recently by Fujino,[1] and by Stotz and co-workers.[2,3] Depending upon their origin, these natural products differ by the substituent RCO– which may be a palmitic, stearic, nervonic, or lignoceric acid residue. In this paper we wish to announce the synthesis of two dihydro derivatives of I, namely, palmitoyldihydrosphingomyelin (VIIIa) and stearoyldihydrosphingomyelin (VIIIb).

For an unambiguous synthesis we chose as key intermediate the oxazoline III, a derivative of dihydrosphingosine in which both the secondary hydroxyl and the amino group are blocked.

Methyl *threo*-α-benzamido-β-hydroxystearate[4] was cyclized with thionyl chloride to *cis*-2-phenyl-4-carbomethoxy-4-pentadecyl-2-oxazoline (II), m.p. 43–45°. (Found: C, 75.38; H, 10.11; N, 3.36.) Reduction with lithium aluminum hydride yielded 85% of the hydroxymethyloxazoline III, m.p. 98–99°. (Found: C, 77.6; H. 10.3; N, 3.0).

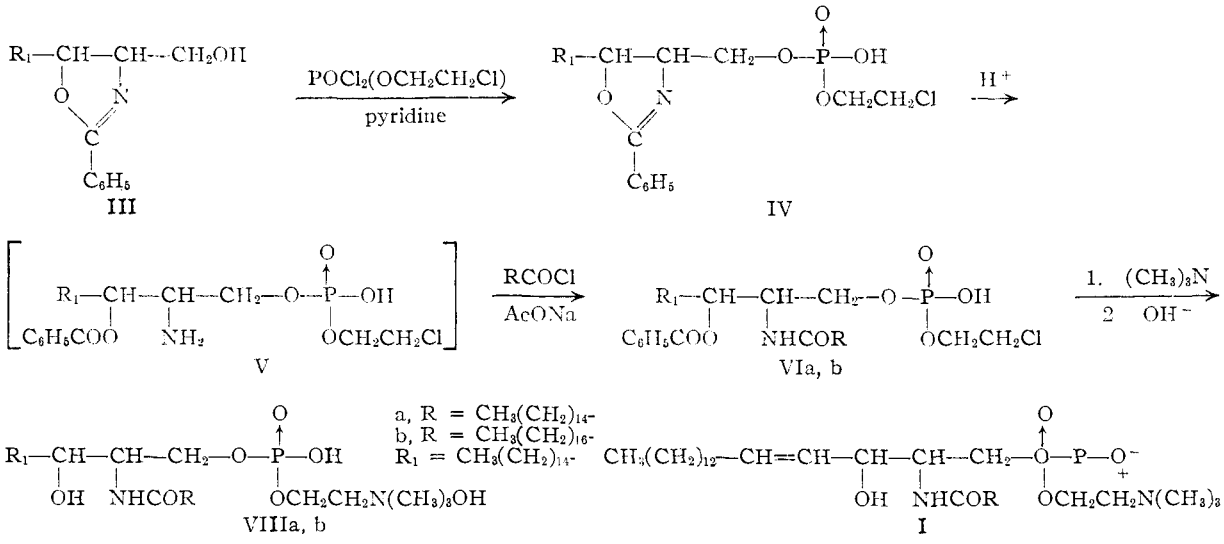
Treatment of III with β-chloroethylphosphoryl dichloride in the presence of pyridine led to the phosphate ester IV which was isolated in pure form as its barium salt (m.p. 143–145°) in a 30%

(1) Y. Fujino, *J. Biochem.* (*Japan*), **39**, 45 (1952).

(2) G. Rouser, J. F. Berry, G. Marinetti and E. Stotz, THIS JOURNAL, **75**, 310 (1953).

(3) G. Marinetti, J. F. Berry, G. Rouser and E. Stotz, *ibid.*, **75**, 313 (1953).

(4) H. E. Carter, J. B. Harrison and David Shapiro, *ibid.*, **75**, 4705 (1953).



yield. (Found: C, 53.95; H, 7.41; N, 2.58; Cl, 6.07; P, 5.1; Ba, 11.6.)

Refluxing with the equivalent amount of hydrochloric acid opened the oxazoline ring to give the *erythro* form of the benzoxy ester V which was not isolated, but was acylated with the corresponding acid chlorides in the presence of sodium acetate. Palmitoyl chloride yielded the amide VIa, m.p. 98–99.5°. (Found: C, 65.75; H, 9.8; N, 1.93; P, 3.6; Cl, 4.58.) Prolonged treatment of the barium salt of VIa with trimethylamine[5,6] gave N - palmitoyl - 3 - O - benzoxydihydrosphingosinephosphorylcholine chloride (VIIa), m.p. 170–175°. (Found: C, 65.39; H, 10.43; N, 3.14; P, 3.77.) Removal of the benzoxy group by mild alkaline hydrolysis afforded palmitoyldihydrosphingomyelin (VIIIa), a hygroscopic compound of m.p. 210–212°. (Found: C, 64.1; H, 12.0; N, 3.83; P, 4.17).

Similarly we prepared stearoyl-dihydrosphingomyelin (VIIIb), m.p. 213–214°. (Found: C, 65.97; H, 12.05; N, 3.89; P, 4.18).

The intermediate compounds were characterized as follows: N-steroyl-3-O-benzoxydihydrosphingosinephosphoryl ethylene chlorohydrin (VIb), m.p. 95–97°. (Found: C, 66.38; H, 10.45; N, 1.82; P, 4.04; Cl, 4.01.) N-stearoyl-3-O-benzoxydihydrosphingosinephosphoryl choline chloride (VIIb), m.p. 170–172°. (Found: N, 3.35; P, 3.77.)

Both dihydrosphingomyelins gave analytical carbon values which correspond closely to the hydrated form of the zwitterionic structure I, and are, therefore, expressed by formula VIII. This result is consistent with the behaviour of the lecithins, recently reported by Baer and co-workers.[7]

The infrared spectra of VIIIa and VIIIb (Nujol) were essentially identical and showed characteristic bands at: 2.94, 3.12, 6.1, 6.41, 6.73, 6.95, 8.13, 9.08, 9.43, 10.28, 10.77, 11.41, 12.02, and 13.94 μ. The absorption peaks reported[8] for the natural product are: 3.10, 6.06, 6.42, 6.75, 7.05, 8.12, 9.18, 9.44, 10.29, 10.86, 11.41, 12.01, 13.09 and 13.86 μ.

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RECEIVED MARCH 14, 1958

(5) A. Gruen and F. Kade, *Ber.*, **45**, 3367 (1912).
(6) Erich Baer, Dmytro Buchnea and Alan G. Newcombe, THIS JOURNAL, **78**, 232 (1956).
(7) Erich Baer, *ibid.*, **75**, 621 (1953).
(8) G. Marinetti and E. Stotz, *ibid.*, **76**, 1347 (1954).

THE INCORPORATION OF 5-FLUOROURACIL INTO THE NUCLEIC ACID OF TOBACCO MOSAIC VIRUS[1]

Sir:

The recent report of the incorporation of 5-fluorouracil (FU) into the nucleic acids of Ehrlich ascites tumor[2] has prompted an investigation of the effect of this compound on the production of tobacco mosaic virus (TMV). Excised disks from Turkish tobacco leaves infected 24 hours previously with the virus were floated on a 0.1% solution of FU. Under these conditions the yield of TMV was reduced by 50% as compared to a water control. The ribonucleic acid (RNA) was prepared[3] from TMV grown in the presence of 0.1% FU. Electrophoresis at *p*H 9.2 of alkaline hydrolysates of the RNA revealed an additional component which migrated more rapidly than the uridylic acids (UA) in a control hydrolysate. The spectrum (λ_{max} 266 mμ, *p*H 7) was consistent with the formulation of the material as a mixture of the 2′ and 3′ phosphates of 5-fluorouridine. The RNA was hydrolyzed with 72% perchloric acid, and the base composition was determined by two dimensional paper chromatography in isopropyl alcohol–hydrochloric acid–water[4] followed by *n*-butyl alcohol–ammonia[5] (Table I).

A FU substituted TMV-RNA labeled with P^{32} was prepared. Separate samples were hydrolyzed with snake venom diesterase and alkali. These hydrolysates were separated into 4 bands by electrophoresis at *p*H 3.5. The bands corresponding

(1) Aided by a grant from the U. S. Public Health Service and by a grant from the National Foundation for Infantile Paralysis.
(2) C. Heidelberger, N. K. Chaudhuri, P. Danneberg, D. Mooren, L. Griesbach, R. Duschinsky, R. J. Schnitzer, E. Pleven and J. Scheiner, *Nature*, **179**, 663 (1957).
(3) C. A. Knight, *J. Biol. Chem.*, **197**, 241 (1952).
(4) G. R. Wyatt, *Biochem. J.*, **48**, 584 (1951).
(5) W. S. MacNutt, *ibid.*, **50**, 384 (1952).