The physiological activity is under study. Preliminary work indicates that the compound has some degree of hypotensive action, and comparison studies are in progress with andromedol, acetylandromedol and the crystalline adduct of andromedol and ethyl acetate.

Andromedol has not been observed in isolation studies with *R. maximum* and *K. angustifolia* var. *caroliniana*, but it might have been present in low concentration in these plants. It has been reported to be present, along with grayanotoxin I (acetylandromedol) in extracts from *L. grayana*.[15]

Anhydroandromedol.—A solution of acetylandromedol (12.5 g.) in 2.5 l. of water containing 7.5 ml. of concd. sulfuric acid was heated under reflux for 4 hr. The dark mixture was neutralized with sodium hydroxide and filtered to remove polymerized material. The filtrate was extracted with chloroform; the chloroform was removed under reduced pressure to yield 3.8 g. of dark, amorphous material. The usual chromatographic methods (alumina, silica) failed to yield a pure substance. Preliminary small-scale studies indicated that purification by countercurrent distribution might be successful. With a solvent system containing equal parts of water, ethanol, ethyl acetate and cyclohexane (this mixture gives two phases of about equal volume) a separation of components was effected. The separation was carried out with 3.6 g., in a 50-tube apparatus with 100 ml. per phase capacity, and with an arrangement for collecting withdrawal fractions of top phase.

The shape of the major band with a peak concentration in tube 37 was compared with a theoretical curve; Fig. 3 shows the experimental and theoretical values. The close correspondence provides evidence for the purity and homogeneity of this product. The contents of tubes 30 to 45 were combined to yield 1.1 g. of crystalline material, m.p. 223–226° after recrystallization from benzene–ethyl acetate.

The infrared spectrum (Nujol) of this compound showed a broad hydroxy band (2.80 μ) but no evidence of a carbonyl group or unsaturation. The ultraviolet absorption was examined to 195 mμ in water solution, but no evidence of unsaturation was found. The compound did not decolorize bromine in chloroform solution, and an ethanol solution did not absorb hydrogen in the presence of a 10% Pd–C catalyst (the compound was recovered unchanged).

The optical rotation was found to be $[\alpha]^{25}_{589}$ −9.3, $[\alpha]^{25}_{436}$ −15.2 (*c* 0.76, ethanol).

When subjected to electrophoresis on paper with a borate solution, the substance gave a purple Godin color and had a mobility of zero. It displayed no physiological activity.

In addition to the analytical data in Table III, the compound was examined for C-CH_3 and active hydrogen.

Anal. Calcd. for $C_{20}H_{32}O_5$: C-CH_3, 8.53 (for two), 12.80 (for three); active H, 0.86 (for three), 1.14 (for four). Found: C-CH_3, 9.84; active H, 1.04.

The broad band with maximum at tube 90 (Fig. 3) was not characteristic of a pure substance; when material from this tube was examined through paper electrophoresis with a borate solution, two compounds were indicated. Both gave purple Godin colors; one was uncharged and the other moved slightly toward the anode. The mixture showed ultraviolet absorption in ethanol solution (λ_{max} 248 mμ) and therefore consisted in part of an unsaturated compound. Tubes 60 to 110 were combined and attempts to separate the components were not successful.

Tubes 125 to 149 contained dark material which was not investigated.

BETHESDA 14, MD.

COMMUNICATIONS TO THE EDITOR

TRANSFER OF THE METHYL GROUP OF METHIONINE TO CARBON-24 OF ERGOSTEROL[1]

Sir:

In a previous communication it was demonstrated that the methyl group of methionine serves as a source of the 24-methyl group of ergosterol in yeast.[2] The mechanism of this reaction remained, however, in doubt. Since formate also has been established as a possible source of the 24-methyl group,[3] the oxidation of methionine-methyl to "active formate" with subsequent reduction provided one possible explanation, using only well established biochemical transformations.[4] On the other hand, while transfers of intact methyl groups from sulfur to nitrogen[4] and oxygen[5] were known, no transmethylation to carbon has been reported.

In our hands, formate, formaldehyde, $NaHCO_3$ and serine-3-C^{14} gave lower C^{14} incorporation than methionine[2] (also see Table I). Almost 6% of formate-C^{14} was incorporated into the non-saponifiable fraction of yeast; however, addition of non-radioactive methionine cut the formate-C^{14} incorporation to 0.3%. Under similar conditions, 24.8% of methionine-methyl-C^{14} was incorporated and addition of non-radioactive formate did not affect this incorporation significantly. Addition of homocysteine, which reacts with formate to give methionine,[6] doubled the yields from formate-C^{14}. Folic acid, which is essential in *de novo* methyl synthesis, further increased the yield. On the other hand, aminopterin, a folic acid antagonist, decreased the formate-C^{14} incorporation to 2.3%. While formate itself had only a slight effect on the incorporation of methionine-methyl-C^{14}, formate and homocysteine cut this incorporation sharply. Aminopterin, which prevents the condensation of formate and homocysteine, counteracted the formate homocysteine effect. In the absence of exogeneous formic acid, aminopterin increased the yields from methionine-methyl-C^{14}, probably by preventing partial oxidation of the S-methyl groups.

Serine-3-C^{14} was 50% as efficient as methionine but twice as efficient as formate as the source of C-28 of ergosterol. Non-radioactive serine raised the yield of C-28 from methionine, possibly by acting as an acceptor of the homocysteine moiety, left after the methyl transfer from methionine to ergosterol. The cystathionine thus formed has been shown to be biologically inactive.[6,7]

When doubly labeled methionine, made by mixing methionine-methyl-C^{14} with methionine-

(1) This work was supported by the U. S. Public Health Service, Grant C321, The Jane Coffin Childs Memorial Fund, and an institutional grant from the American Cancer Society.

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(3) H. Danielson and K. Bloch, *ibid.*, **79**, 500 (1957).

(4) G. Ehrensvard, *Ann. Rev. Biochem.*, **24**, 275 (1955).

(5) C. S. Sato, R. U. Byerrum and C. D. Ball, *J. Biol. Chem.*, **224**, 717 (1957).

(6) P. Berg, *ibid.*, **205**, 145 (1953).

(7) V. M. Doctor, T. L. Patton and J. Awapara, *Arch. Biochem. Biophys.* **67**, 404 (1957).

TABLE I

FORMATE-C^{14}, METHIONINE-METHYL-C^{14} AND SERINE-3-C^{14} AS C-24 METHYL DONORS[a]

Source of C^{14}	Non-radioactive additions	Non-saponifiable fraction Mg.	S. A. $\times 10^{-3}$	Total counts $\times 10^{-3}$	Yield, %
HCOOH	...	3.7	2.3	8.6	6
HCOOH	Homocysteine[b]	3.5	4.3	15.2	10
HCOOH	Homocysteine, folic acid	4.0	6.4	25.7	17
HCOOH	Homocysteine, aminopterin	4.2	0.8	3.5	2
HCOOH	Methionine	3.8	0.1	0.4	0.3
Methionine	...	3.9	9.5	37.2	25
Methionine	Formate	5.2	6.1	31.5	22
Methionine	Formate, homocysteine	3.3	2.5	8.5	6
Methionine	Formate, homocysteine, aminopterin	3.7	6.2	20.3	14
Methionine	Aminopterin	1.9	34.4	65.4	44
Methionine	Serine	3.6	28.4	102.1	68
Serine	...	3.5	4.7	16.6	11

[a] Each flask contained 4 ml. of homogenate made from 1 g. of dry yeast, 2×10^{-4} *M* ATP and 0.5 μc. of C^{14} (1 mc./mmole). [b] Additions per flask (where indicated): homocysteine, 5 mg.; folic acid, 1 mg.; aminopterin, 2 mg.; methionine, 15 mg.; HCOONa, 5 mg.; serine, 15 mg.

TABLE II

TRITIUM:CARBON^{14} RATIOS IN METHIONINE AND ERGOSTEROL

Each experiment consisted of 5 flasks, each containing 4 ml. of yeast extract, 5 mg. of serine, 2 mg. of aminopterin, 1 mg. of ATP, and 0.175 mg. of doubly labeled methionine. Incubation time was 48 hr.; radioactivities were determined on a Packard Tri-Carb Scintillation Counter. The values given are the averages of four samples.

Expt.	T:C^{14} Ratio Methionine	Ergosterol
1	1.12 ± 0.06	0.97 ± 0.06
2	1.12 ± 0.06	1.02 ± 0.02

methyl-T, was incubated with yeast homogenates and the resulting ergosterol rigorously purified, the T:C^{14} ratios of the substrate and product showed that all three hydrogen atoms of the methyl group of methionine are transferred to ergosterol. Were the methyl group oxidized to the level of formaldehyde, one third of the tritium would be lost, and the T:C^{14} ratio in ergosterol (Table II) would have been 67%. Since the ratio is 86–91%, at least some methyl groups of methionine must have been transferred intact to the carbon 24 of the sterol.

WORCESTER FOUNDATION FOR EXPERIMENTAL BIOLOGY
SHREWSBURY, MASS. GEORGE J. ALEXANDER
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RECEIVED JULY 2, 1957

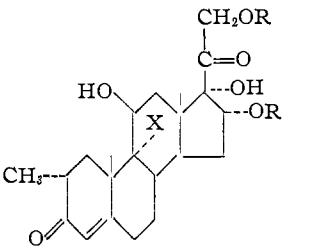
16-HYDROXYLATED STEROIDS. V.[1] THE SYNTHESIS OF THE 16α-HYDROXY DERIVATIVES OF 2α-METHYL-STEROIDS

Sir:

In view of our previous Communication[1] concerning the ability of the 16α-hydroxyl group to abolish the sodium retaining property of a steroid without destroying its glucocorticoid activity, we have investigated the effect of 16α-hydroxylation on the activities of 2α-methyl steroids.[2]

Hydrolysis of 21-acetoxy-3,20-bis-ethylenedioxy-5-pregnene-11β,16α,17α-triol (I)[3] in dilute acetic acid afforded 21-acetoxy-20-ethylenedioxy-11β,16α,17α-triol-4-pregnen-3-one (II), m.p. 262–263°, $[\alpha]^{25}D$ + 85° ($CHCl_3$); (*Anal.* Found: C, 64.77; H, 8.03). Treatment of compound II with ethyl oxalate and sodium methoxide in *t*-butyl alcohol formed the sodium enolate of 20-ethylenedioxy-2-ethoxyoxalyl-11β,16α,17α,21-tetrahydroxy-4-pregnen-3-one (III) as a pale yellow amorphous solid. Methylation of III with methyl iodide and potassium carbonate in acetone followed by removal of the ethoxyoxalyl group by sodium methoxide in methanol gave a glass. After removal of the 20-ketal group with dilute ethanolic sulfuric acid, partition chromatography[4] yielded 11β,16α,17α,21-tetrahydroxy-2α-methyl-4-pregnene-3,20-dione (IVa) apparently with one molecule of acetone of crystallization, m.p. 201–203°, λ_{max}. 240–241 mμ (ε 16,600),[5] $[\alpha]^{25}D$ + 145° ($CHCl_3$); (*Anal.* Found: C, 65.59; H, 8.39). Acetylation gave the 16α,21-diacetate IVb, m.p. 253–254°, λ_{max}. 240–241 mμ (ε 17,500), ν_{max}^{KBr} 3450, 1743, 1726 (shoulder), 1654, 1620 and 1238 cm.$^{-1}$, $[\alpha]^{25}D$ + 92° ($CHCl_3$); (*Anal.* Found: C, 65.43; H, 7.75). Oxidation of IVb with chromium trioxide–pyridine reagent[6] gave 16α,21-diacetoxy-17α-hydroxy-2α-methyl-4-pregnene-3,11,20-trione (V), m.p. 240.5–241.5°, $[\alpha]^{25}D$ + 129° ($CHCl_3$); (*Anal.* Found: C, 65.49; H, 7.30).

IVa, X = H, R = H
IVb, X = H, R = Ac
XIIIa, X = F, R = H
XIIIb, X = F, R = Ac



Acetylation of 3,20-bis-ethylenedioxy-5-pregnene-11β,16α,17α,21-tetrol (VIa)[7] yielded the 16α,21-diacetate (VIb), m.p. 129–135°,[8] $[\alpha]^{25}D$ − 61.5° ($CHCl_3$); (*Anal.* Found: C, 62.49; H, 7.81). Treatment with phosphorus oxychloride in pyridine afforded 3,20-bis-ethylene-dioxy-16α,21-diacetoxy-5,9(11)-pregnadien-17α-ol (VII), m.p. 221–224°, $[\alpha]^{25}D$ − 48° ($CHCl_3$); (*Anal.* Found: C, 65.52; H, 7.67). Hydrolysis of VII in dilute acetic acid gave 16α,21-diacetoxy-20-ethylenedioxy-17α-hydroxy-4,9(11)-pregnadien-3-one (VIII), m.p. 184.5–186°, $[\alpha]^{25}D$ ± 0° ($CHCl_3$); (*Anal.* Found: C, 66.63; H, 7.65).

The sodium enolate (IX) of the 2-ethoxyoxalyl

(1) Paper IV, S. Bernstein, R. H. Lenhard, W. S. Allen, M. Heller, R. Littell, S. M. Stolar, L. I. Feldman and R. H. Blank, THIS JOURNAL, **78**, 5693 (1956).

(2) J. A. Hogg, F. H. Lincoln, R. W. Jackson and W. P. Schneider, *ibid.*, **77**, 6401 (1955).

(3) W. S. Allen and S. Bernstein, *ibid.*, **78**, 3223 (1956).

(4) R. Littell and S. Bernstein, *ibid.*, **78**, 984 (1956).

(5) The ultraviolet spectra were determined in absolute alcohol solutions.

(6) G. I. Poos, G. E. Arth, R. E. Beyler and L. H. Sarett, THIS JOURNAL, **75**, 422 (1953).

(7) W. S. Allen and S. Bernstein, *ibid.*, **78**, 1909 (1956).

(8) This compound seemed to be solvated and could not be brought to a better melt or analytical value.