Hydroxylation of 3-Methoxy-H³ dopamine[4]. The deproteinized incubation mixture which contained 3-methoxy-H³ dopamine as a substrate was adjusted to pH 4 and the amines present in the mixture were separated as previously described[6]. The acetylated amines were chromatographed in the Bush C solvent system and were scanned for radioactive zones. Two radioactive zones were detected. One radioactive zone had the same mobility as acetylated 3-methoxy dopamine, and the other as acetylated 3-methoxy norepinephrine. The radioactive zone with the same mobility as 3-methoxy-norepinephrine was eluted and diluted with non-radioactive acetylated 3-methoxy norepinephrine and rechromatographed in the Bush C solvent system. All the radioactivity was found to be associated with acetylated 3-methoxy norepinephrine.

Hydroxylation of Hydroxyamphetamine. The amines present in the deproteinized incubation mixture which contained hydroxyamphetamine as a substrate were acetylated and subsequently chromatographed in the Bush C solvent system. A spot having the same Rf value and yielding the same color with diazotized sulfanilic acid as authentic *p*-hydroxyphenyl propanolamine was obtained (Table).

Chromatographic separation of substrate from enzymatic formed β-hydroxylated product

Substrate	Product	Rf value		Color reaction on paper	
		Substrate	Product	Substrate	Product
Phenylethyl amine	phenyl-ethanol amine (α-phenyl-β-amino ethanol)	0.63[a]	0.55[a]	brown	blue
3-methoxy-H³ dopamine	3-methoxy-H³ norepi-nephrine	0.9[b]	0.42[b]	—	—
dl-p-hydroxy-α-methyl-phenylethyl-amine (*p*-hydroxy-amphetamine)	*dl-p*-hydroxy-α-methyl-phenyl-β-amino ethanol	0.75[b]	0.31[b]	red	yellow

[a] The solvent system was butanol acetic acid water.
[b] The acetylated compounds were chromatographed in the 'C' solvent system of BUSH[10].

The *in vitro* β-hydroxylation of phenylethylamine, 3-methoxy dopamine and *p*-hydroxyamphetamine may also occur *in vivo* and represent an important metabolic path for these compounds. It is possible that the pharmacological activity of phenylethylamine and *p*-hydroxyamphetamine is a result of the β-hydroxylation of these compounds to the corresponding hydroxy derivatives. In this connection it may be interesting to report that rats pretreated with ipronazid and then treated with phenylethanolamine or with phenylethylamine show severe excitation.

Although the conversion of 3-methoxy dopamine to 3 methoxy-norepinephrine occurs to a lesser degree than the conversion of dopamine to norepinephrine[7], it is possible that some of the 3-methoxy norepinephrine formed *in vivo* derives from the 3-methoxy dopamine and not as previously assumed only from norepinephrine.

Since it has been previously shown that amphetamine like *p*-hydroxyamphetamine inhibits the conversion of dopamine to norepinephrine[8] it may be assumed therefore that amphetamine is also a substrate of dopamine β-oxidase.

Finally, the present findings show that a quinoid structure is not a requirement for enzymic β-hydroxylation, and therefore, in its biogenesis norepinephrine need not pass through a quinoid intermediate as previously postulated[9].

Zusammenfassung. Phenylethylamin, 3-Methoxy-dopamin und *p*-Hydroxyamphetamin werden durch Dopamin-β-oxidase in die entsprechende β-Hydroxy-Verbindung umgewandelt.

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Department of Psychiatry and Neurology, Neurochemistry Laboratory, New York University College of Medicine, New York, May 2, 1961.

[3] E. Y. LEVIN et al., J. biol. Chem. *235*, 2080 (1960).
[4] The 3-methoxy-H³ dopamine was prepared enzymatically by the method of J. AXELROD et al.[5].
[5] J. AXELROD et al., J. biol. Chem. *233*, 697 (1958).
[6] M. GOLDSTEIN et al., Proc. Soc. exp. Biol. Med. *103*, 137 (1960).
[7] M. GOLDSTEIN et al., to be published.
[8] M. GOLDSTEIN et al., Biochem. Pharmacol., in press.
[9] S. SENOH et al., J. Amer. chem. Soc. *81*, 6236 (1959).
[10] I. E. BUSH, Biochem. J. *50*, 370 (1951).

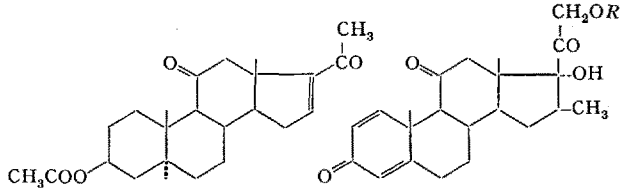
16-β-Methylprednisone from Hecogenin

On the basis of recent reports[1–3], we have studied a new synthetic way to obtain 17-hydroxy-16β-methyl-5α-pregnane derivatives in order to prepare *16β-methylprednisone* starting from 5α-pregn-16-en-3β-ol-11, 20-dione acetate (I), easily available from hecogenine[4–6].

Compound I was methylated at C-16 both with diazomethane (through the intermediate pyrazoline) and with a new method[7,8]. The 16-methyl-5α-pregn-16-en-3β-ol-11, 20-dione acetate treated with H_2O_2 in alkaline medium yielded the 16α, 17α-epoxy derivative (II), m.p. 178–183°, $[\alpha]_D$ + 88.5° ($CHCl_3$), + 79.5° (dioxane); *Anal.* calc. for $C_{22}H_{32}O_4$: C 73.3; H 8.95; found: C 73.51; H 8.99. When reacted upon with *p*-toluenesulfonic acid in benzene, II rearranged into 16-methylene-5α-pregnan-3β, 17α-diol-11, 20-dione (III), m.p. 273–278°, $[\alpha]_D$ – 10.2° (CH_3OH:$CHCl_3$ 1:1), *Anal.* calc. for $C_{22}H_{32}O_4$: C 73.3; H 8.95; found: C 73.30; H 8.81. Hydrogenation of III with 5% palladium on calcium carbonate in methanol gave 16β-methyl-5α-pregnan-3β, 17α-diol-11, 20-dione (IV), m.p. 260–266°, $[\alpha]_D$ + 71° (dioxane); *Anal.* calc. for $C_{22}H_{34}O_4$: C 72.89;

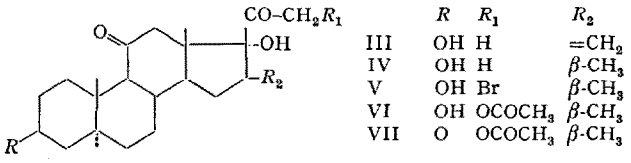
[1] D. TAUB, R. D. HOFFSOMMER, H. L. SLATES, C. H. KUO, and N. L. WENDLER, J. Amer. chem. Soc. *82*, 4012 (1960).
[2] G. NOMINÉ, D. BERTIN, and A. PIERDET, Tetrahedron *8*, 217 (1960).
[3] D. N. KIRK, V. PETROW, M. STANSFIELD, and D. M. WILLIAMSON, J. chem. Soc. *1960*, 2385.
[4] E. M. CHAMBERLAIN, W. V. RUYLE, A. E. ERICKSON, J. M. CHEMERDA, L. M. ALIMINOSA, R. L. ERICKSON, G. E. SITA, and M. TISHLER, J. Amer. chem. Soc. *73*, 2396 (1951).
[5] C. DJERASSI, E. BATRES, J. ROMO, and G. ROSENKRANZ, J. Amer. chem. Soc. *74*, 3634 (1952).
[6] A. F. B. CAMERON, R. M. EVANS, J. C. HAMLETT, J. S. HUNT, P. G. JONES, and A. G. LONG, J. chem. Soc. *1955*, 2807.
[7] P. DE RUGGIERI, Farmaco, Ed. sci. *16*, 58 (1961).
[8] K. HEUSLER, J. KEBRLE, C. MEYSTRE, H. UEBERWASSER, P. WIELAND, G. ANNER, and A. WETTSTEIN, Helv. chim. Acta *42*, *42*, 2043 (1959).

H 9.45; found: C 73.15; H 9.40. Under suitable experimental conditions (neutral pH, slow hydrogenation rate) the formation of the 16α-methyl-isomer may be completely avoided. A chloroform suspension of IV, in the presence of a small amount of methanol, was treated with bromine and hydrobromic acid to give *21-bromo-16β-methyl-5α-pregnan-3β,17α-diol-11,20-dione* (V), m.p. 220 to 223°, $[\alpha]_D$ + 90.1° (dioxane); *Anal.* calc. for $C_{22}H_{33}BrO_4$: C 59.86; H 7.54; Br 18.1; found: C 59.70; H 7.43; Br 18.38. Potassium acetate in refluxing acetone converted V to *16β-methyl-5α-pregnan-3β,17α,21-triol-11,20-dione 21-acetate* (VI), m.p. 223–227°, $[\alpha]_D$ + 95.8° (dioxane); *Anal.* calc. for $C_{24}H_{36}O_6$: C 68.58; H 8.63; found: C 68.06; H 8.54. Chromic acid in acetone[9] or chromic anhydride in acetic acid or N-bromoacetamide oxidized VI to *16β-methyl-5α-pregnan-17α,21-diol-3,11,20-trione-21-acetate* (VII), m.p. 210–213°, $[\alpha]_D$ + 120° (dioxane); *Anal.* calc. for $C_{24}H_{34}O_6$: C 68.9; H 8.19; found: C 68.3; H 7.82. Compound VII, when treated with bromine in dioxane, yielded the 2,4-dibromo-derivative (VIII), which, as crude product, was dehydrobrominated in dimethylformamide solution[10] to give *16β-methylprednisone-21-acetate* (*IX*), m.p. 224–229°, $[\alpha]_D$ + 210° (dioxane); λ_{max} 238 mμ, $E^{1\%}_{1\,cm}$ 358 (methanol). IX was de-acetylated by conventional methods to *16β-methylprednisone* (*X*), m.p. 200–205°. $[\alpha]_D$ + 200° (dioxane), $E^{1\%}_{1\,cm}$ 416 at 239 mμ in methanol. IX and X are identical with authentic specimens.



I

IX $R = COCH_3$
X $R = H$



	R	R_1	R_2
III	OH	H	$=CH_2$
IV	OH	H	β-CH_3
V	OH	Br	β-CH_3
VI	OH	$OCOCH_3$	β-CH_3
VII	O	$OCOCH_3$	β-CH_3

Zusammenfassung. Es wird über die Herstellung des 16-Methylprednisons berichtet, welche vom Hecogenin ausgehend über ca. 15 Stufen durchgeführt wird. Als wichtige Zwischenstufe treten 5α-Pregn-16-en-3β-ol-11, 20-dion-Acetat und 16-Methylen-5α-pregnan-3β,17α-diol-11,20-dion auf: letzteres wird durch eine stereospezifische katalytische Reduktion in das entsprechende 16β-Methylderivat umgewandelt.

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Research Laboratories of Lepetit S.p.A., Milano (Italy), June 26, 1961.

[9] K. Bowden, I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, J. chem. Soc. *1946*, 39.

[10] R. Joly, J. Warnant, G. Nominé, and D. Bertin, Bull. Soc. chim. Fr. [5] *15*, 366 (1958).

Conversion of 17α-Hydroxypregnenolone to Cortisol

In the commonly accepted scheme of adrenal corticoid biosynthesis, cortisol is considered to arise from the conversion of pregnenolone (3β-hydroxy-pregn-5-en-20-one) to progesterone, which is then successively hydroxylated to cortisol. It has been demonstrated[1], however, that pregnenolone can undergo initial 17α-hydroxylation, resulting in the formation of 17α-hydroxypregnenolone, and this product has been isolated from dog adrenal vein blood[2]. It has been proposed[3] that 17α-hydroxypregnenolone is the precursor of dehydroepiandrosterone (DHA), either in the adrenal or elsewhere, and this conversion has been shown to occur in a patient with adrenal cancer[4] and in particulate fractions of beef adrenal and testis[1]. We have examined the metabolism of 17α-hydroxypregnenolone and have found that it is converted to 17α-hydroxyprogesterone, 11-deoxycortisol and cortisol by adrenal slices from the human, guinea pig and rat.

Experimental. 17α-hydroxypregnenolone-7-H^3 (New England Nuclear Corp.) was diluted with stable 17α-hydroxypregnenolone and chromatographed in the system toluene/propylene glycol. Material from the major peak contained 93% of the radioactivity and had a specific activity of 2.9×10^8 cpm/mg. Pregnenolone-7-H^3 (New England Nuclear Corp.) was diluted with stable pregnenolone and chromatographed in the system Bush B_3. The major peak contained 89% of the radioactivity and had a specific activity of 1.8×10^8 cpm/mg. Tritium and C^{14} counting were performed in the Packard Tri-Carb Scintillation spectrometer at an approximate efficiency of 15% for tritium and 80% for carbon[14].

Incubations were performed in pH 7.4 saline-phosphate buffer[5] for 3 h under air at 37°C. The human adrenals were obtained from two women undergoing adrenalectomy for breast cancer. The rats and guinea pigs were killed by decapitation.

Human adrenal slices with total weights of 3.1 g and 5.5 g were incubated with 5.2×10^6 cpm and 1.3×10^7 cpm respectively of 17α-hydroxypregnenolone. Similarly 3.1 g of slices were incubated with 2.8×10^6 cpm of pregnenolone. A total of 504 mg of guinea pig adrenal slices and 374 mg of rat adrenal slices were each incubated with 5.2×10^6 cpm of 17α-hydroxypregnenolone. After incubation, 200 γ of the following steroids were added: cortisol, 11-deoxycortisol, 17α-hydroxyprogesterone, progesterone, corticosterone and DHA. The contents of the flask were extracted with 80% acetone, defatted in cold 70% methanol, and partitioned between hexane and 70% methanol. The 3β-hydroxysteroids were separated with digitonin. The non-digitonin-precipitable steroids were initially chromatographed in chloroform/formamide. Position of the compounds was determined by UV absorption and the Zimmerman reaction, and radioactivity by scanning on a Baird-Atomic strip scanner. Evidence

[1] F. W. Kahnt, R. Neher, K. Schmid, and A. Wettstein, Exper. *17*, 1 (1961).

[2] H. Carstensen, G. W. Oertel, and K. Eik-Nes, J. biol. Chem. *234*, 2570 (1959).

[3] S. Lieberman and S. Teich, J. clin. Endocrin. *13*, 1140 (1953).

[4] S. Solomon, A. C. Carter, and S. Lieberman, J. biol. Chem. *235*, 351 (1960).

[5] J. R. Robinson, Biochem. J. *45*, 68 (1949).