of the names, hydroxylysine and allohydroxylysine, to the pure isomers has been made.

LABORATORY OF BIOCHEMISTRY
NATIONAL CANCER INSTITUTE
NATIONAL INSTITUTES OF HEALTH[15]
BETHESDA 14, MARYLAND
WILLIAM S. FONES

RECEIVED AUGUST 17, 1953

(15) U. S. Public Health Service, Department of Health, Education and Welfare.

LOW MOLECULAR WEIGHT DEXTRAN AS A MODIFIER OF DEXTRAN SYNTHESIS[1]

Sir:

Dextran synthesis by *Leuconostoc mesenteroides* is brought about by a special enzyme, dextransucrase, that apparently causes the direct transfer of α-D-glucopyranosyl radicals from many sucrose molecules to a few acceptor molecules which become growing dextran chains.[2] Following the report of Koepsell, *et al.*,[3] that certain sugars can serve as "alternate" acceptors for dextransucrase, we[4] and independently Tsuchiya, Hellman and Koepsell[5] have found that low molecular weight dextrans also serve as acceptors. An especially significant point revealed by our experiments is that small-sized dextrans are intrinsically much more potent modifiers of dextran synthesis than such sugars as maltose or glucose.

To compare the modifying action of dextrans and sugars, dextransucrase from *L. mesenteroides* strain B[2] was incubated at 25° and *p*H 5.0 with sucrose (50 mg./ml. final concentration) and the substance to be tested as a supplement. Individual controls were prepared, comprising enzyme and sucrose incubated together, with the supplementary substance added after incubation. The final mixtures were analyzed for dextran and oligosaccharide contents (Fig. 1).

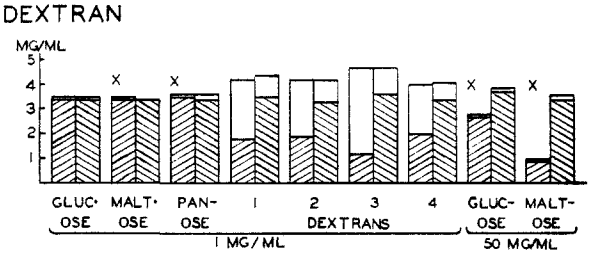


Fig. 1.—Low molecular weight dextrans *vs.* sugars as modifiers of dextran synthesis. Analyses of incubated enzyme–sucrose–supplement mixtures shown at the left of analyses of control enzyme–sucrose mixtures with the supplement added after incubation. Hatched area show dextran precipitated at 25° by 40% (v./v.) methanol; open areas show dextran precipitated between 40 and 65% methanol; × indicates oligosaccharide(s), other than sucrose or supplement, detected by chromatography.[3] Dextrans 1 and 2 were clinical products, kindly supplied by Commercial Solvents Corp., Terre Haute, Ind., and Dextran Corp., Yonkers, N. Y.; dextrans 3 and 4 were fractions of mol. wt. *ca.* 20,000 and 18,000 kindly supplied by Dr. F. R. Senti and Dr. B. Ingelman.

At 1 mg./ml., glucose as a supplement had no detectable effect on the dextran polymerization, while maltose and crystalline panose (kindly supplied by Dr. S. C. Pan) induced formation only of traces of oligosaccharides. "Normal" dextran, precipitable by 40% methanol, was synthesized to the same extent in the presence as in the absence of the three sugars. In contrast, each of four different low molecular weight dextrans (actually fractions of partly hydrolyzed dextrans) profoundly affected the synthesis. Mixtures incubated with these supplements contained smaller amounts of dextran precipitated by 40% methanol than the controls, and had appreciable contents of presumably lower molecular weight dextran, precipitating between 40 and 65% methanol. In the control mixtures, most of the 40 to 65% fraction represents added dextran supplement.

The modifying effect of the 1 mg./ml. dextran supplements was greater than that of 50 mg./ml. glucose and, in at least one instance, approached that caused by 50 mg./ml. maltose. Taking molecular concentration into account, it is evident that the acceptor capacity of an individual small dextran molecule is exceedingly high and of a different order of magnitude than the sugars tested. Attention is directed to this capacity as one factor that may enable useful modifications of dextran synthesis to be made.

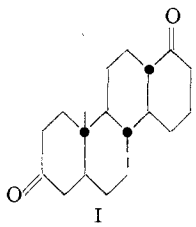
DEPARTMENT OF BACTERIOLOGY AND IMMUNOLOGY
CORNELL UNIVERSITY MEDICAL COLLEGE
NEW YORK, N. Y.
EDWARD J. HEHRE

RECEIVED AUGUST 20, 1953

18-NOR-D-HOMOANDROSTANE-3,17a-DIONE

Sir:

The (±) diketone I, m.p. 149–150.5° cor. (Found: C, 79.0; H, 9.91) has been prepared by chromic acid oxidation of 18-nor-D-homoepiandrosterone which, as already reported,[1] is readily made in five operations from 2,5-dimethoxynaphthalene.



I

Androgenic assays in rats performed under the direction of Drs. R. K. Meyer and Elva G. Shipley of the Department of Zoölogy show this racemic compound to be at least one-tenth as active as testosterone. Since androstane-3,17-dione itself

(1) Supported by a grant from the Corn Industries Research Foundation.

(2) E. J. Hehre, *Proc. Soc. Exp. Biol. Med.*, **54**, 240 (1943); *J. Biol. Chem.*, **163**, 221 (1946); "Advances in Enzymology," Interscience Publishers, Inc., New York, N. Y., **11**, 297 (1951).

(3) H. J. Koepsell, H. M. Tsuchiya, N. N. Hellman, A. Kazenko, L. A. Hoffman, E. S. Sharpe and R. W. Jackson, *Bact. Proc.*, 24 (1952); *J. Biol. Chem.*, **200**, 793 (1953).

(4) E. J. Hehre, Amer. Chem. Soc., Abstracts of Papers for 122nd Meeting, 18A (1952).

(5) H. M. Tsuchiya, N. N. Hellman and H. J. Koepsell, THIS JOURNAL, **75**, 757 (1953).

(1) W. S. Johnson, B. Bannister, B. M. Bloom, A. D. Kemp, R. Pappo, E. R. Rogier and J. Szmuszkovicz, THIS JOURNAL, **75**, 2275 (1953).

does not exhibit more than one-tenth the activity of testosterone in rats,[2] the (+) enantiomer of I thus would appear to be somewhat more potent than its natural congener.

To our knowledge this represents the first report of physiological tests on a true 18-nor-D-homo steroid, and the activity of I lends support to the hypothesis of Birch,[3] also entertained by us independently, that the 18-nor hormones may be expected to exhibit activity provided the C/D rings remain *trans*-fused, a condition which, in the case of 17-keto compounds, is more apt to be satisfied with a 6- than with a 5-membered ring D. The diketone I, moreover, represents the first reported totally synthetic androgenic substitute of any appreciable activity.[4]

The above observation has prompted us to initiate an extensive program to prepare and study the physiological properties of the 18-nor-D-homosteroids in both the natural and unnatural stereochemical series, including the 11-oxygenated derivatives.

(2) E. Tschopp, *Arch. Intern. pharmacodynamie*, **52**, 381 (1935).
(3) A. J. Birch and J. A. K. Quartey, *Chem. and Industry*, 489 (1953).
(4) *Cf.* review of field, ref. 3.

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN
WILLIAM S. JOHNSON
HENRY LEMAIRE
RAPHAEL PAPPO

RECEIVED SEPTEMBER 8, 1953

ALKALOIDS OF RAUWOLFIA SERPENTINA BENTH I. THE CHARACTERIZATION OF RESERPINE AND ITS HYDROLYSIS PRODUCTS

Sir:

The sedative principle of *Rauwolfia serpentina* Benth has previously been reported by Dutt, *et al.*,[1] as residing in the so-called "oleoresin fraction." Recently, Muller, Schlittler and Bein[2] announced the isolation of a new crystalline alkaloid reserpine from this fraction to which they ascribed the sedative action. In a further communication[3] dealing with the pharmacology of reserpine it was revealed that this alkaloid possessed hypotensive activity.

In an independent investigation of the physiologically active constituents of *Rauwolfia serpentina* Benth we have isolated reserpine[4] in sufficient quantity to establish its empirical formula and to subject it to preliminary structural investigation.

Reserpine was obtained by chromatographing the "oleoresin fraction"[1] on a silicic acid-celite column. The alkaloid crystallized from methanol as flat, colorless needles, m.p. 252° (dec.),[5] $[\alpha]^{24}D$ −122 (*c* 1.0 in $CHCl_3$). The analytical data were in agreement for the empirical formula $C_{35}H_{44}O_{10}N_2$:[6] Calcd. C, 64.40; H, 6.80; N, 4.29; 6 OCH_3, 28.53; Found: C, 64.67; H, 6.49; N, 4.33; OCH_3, 28.49. The compound showed the absence of an N–CH_3 group. Sulfate, m.p. 258°, $C_{35}H_{44}O_{10}N_2 \cdot H_2SO_4$; Calcd. C, 55.99; H, 6.18; N, 3.73; S, 4.27; 6 OCH_3, 24.80; Found: C, 56.08; H, 6.06; N, 3.52; S, 4.62; OCH_3, 24.83; hydrochloride monohydrate, m.p. 220°. $C_{35}H_{44}O_{10}N_2 \cdot HCl \cdot H_2O$; Calcd. C, 59.52; H, 6.71; N, 3.97; Cl, 5.02; Found: C, 59.64; H, 6.81; N, 4.05; Cl, 5.07; perchlorate, m.p. 257°. $C_{35}H_{44}O_{10}N_2 \cdot HClO_4$; Calcd. C, 55.81; H, 6.02; N, 3.72; Cl, 4.71; Found: C, 55.95; H, 5.99; N, 3.77; Cl, 4.87.

On subjecting reserpine to hydrolysis with 0.75 *N* methanolic sodium hydroxide, *reserpinolic acid* and 3,4,5-trimethoxybenzoic acid were recovered. The 3,4,5-trimethoxybenzoic acid (m.p. 167–168°) gave no depression of melting point on admixture with an authentic sample. The infrared spectra were identical. Calcd. for $C_{10}H_{12}O_5$: C, 56.60; H, 5.70; Found: C, 56.61; H, 5.72. Reserpinolic acid melted at 240°; $[\alpha]^{24}D$ −70 ± 3 (*c* 0.97 in H_2O).[7] The analytical data were in agreement for an empirical formula of $C_{24}H_{32}O_6N_2$; Calcd. for $C_{24}H_{32}O_6N_2 \cdot 2H_2O$: C, 59.98; H, 7.55; 2 OCH_3, 12.92; Found: (dried at 66° (2 mm.)) C, 60.48; H, 7.28; OCH_3, 13.51; Calcd. for $C_{24}H_{32}O_6N_2 \cdot H_2O$: C, 62.32; H, 7.41; Found: (dried at 125° (2 mm.)) C, 62.45; H, 7.23. Hydrochloride, m.p. 250–253° (dec.). Calcd. for $C_{24}H_{32}O_6N_2 \cdot HCl$: C, 59.93; H, 6.92; 2$OCH_3$, 12.91. Found: C, 60.05; H, 7.06; OCH_3, 13.51. On refluxing reserpinolic acid in methanol and hydrogen chloride, the methyl ester was obtained, m.p. 229–231° (dec.). Calcd. for $C_{25}H_{34}O_6N_2$: C, 65.48; H, 7.47; Found: C, 65.54; H, 7.50. Absorption spectrum: ultraviolet $\lambda_{max.}^{alc.}$ (log ϵ): 222 mμ (4.6), 270 mμ (3.78), 295 mμ (3.86): $\lambda_{max.}^{alc.}$ (log ϵ): 252 mμ (3.74), 277 mμ (3.72).

Reserpine is therefore a diester containing a carbomethoxy group and a hydroxyl esterified with 3,4,5-trimethoxybenzoic acid. The failure of reserpine to acetylate on refluxing with acetic anhydride–pyridine, and the presence of the characteristic NH band (2.9 μ) in the infrared spectrum, is indicative of a tertiary nitrogen and an indifferent secondary nitrogen in the molecule analogous to the indole alkaloids previously shown to be present in this species. Further evidence pointing to the presence of an indole nucleus is found in the parallelism of the color reactions with those of yohimbine; in addition, the similarity in the ultraviolet absorption spectrum of reserpinolic acid and 5,6-dimethoxyindole[8] indicates the site of attachment of the two remaining methoxyl groups. The nature of one of the oxygen atoms in reserpine has not yet been established; however, the presence of an enol ether group could account for its apparent inertness and for the sharp band at 0.17 μ in the infrared spectrum. Further work on the structure of reserpinolic acid is in progess.

RIKER LABORATORIES, INC.
LOS ANGELES, CALIFORNIA
M. W. KLOHS
M. D. DRAPER
F. KELLER
F. J. PETRACEK

RECEIVED AUGUST 12, 1953

(1) Ashutosh Dutt, J. C. Gupta, Sudhamoy Ghosh and B. S. Kahali, *Indian J. Pharm.*, **9**, 54 (1947).
(2) J. M. Muller, E. Schlittler and H. J. Bein, *Experientia*, **8**, 338 (1952).
(3) H. J. Bein, *ibid.*, **9**, 107 (1953).
(4) We wish to thank Dr. E. Schlittler for confirming the identity of our sample of reserpine.
(5) By inserting the melting point tube in the bath at progressively higher temperatures, the melting point could be raised to 263–264°. Muller, *et al.*, reported a melting point of 262–263°.
(6) The analytical data for reserpine would also allow the empirical formula $C_{36}H_{42}O_{10}N_2$; however, the data for reserpinolic acid and its derivatives favor the formula $C_{35}H_{44}O_{10}N_2$.
(7) The physical constants were determined on the dihydrate of reserpinolic acid.
(8) H. S. Mason, *J. Biol. Chem.*, **172**, 83 (1948).