Experimental[2]

2,7-Dihydroxynaphthalene.—This compound was prepared in 43% yield from disodium naphthalene-2,7-disulfonate by the procedure of Chakravarti and Pasupati.[3] The material so obtained was purified by the method of Johnson, Gutsche and Banerjee.[4]

Decalin-2,7-diol.—Hydrogenation of 30 g. (0.0176 mole) of purified 2,7-dihydroxynaphthalene dissolved in 100 ml. of absolute ethanol with W-4 Raney nickel catalyst[5] was carried out in the usual manner[4] at 150° and between 63 and 170 atm. with the latter the initial pressure. Hydrogen uptake ceased after 5 hours. The colorless residue remaining after removal of the catalyst and solvent was taken up in 150 ml. of dry ether and the solution cooled overnight in a refrigerator. The colorless solid which separated weighed 20.1 g. (63%), m.p. 108–110°.

Anal. Calcd. for $C_{10}H_{18}O_2$: C, 70.55; H, 10.66. Found: C, 70.82; H, 10.31.

The dibenzoate, obtained by treatment with benzoyl chloride, was recrystallized from 50% ethanol–water and melted at 102.5–103.5°.

Anal. Calcd. for $C_{24}H_{26}O_4$: C, 76.19; H, 6.87. Found: C, 76.17; H, 6.79.

Decalin-2,7-dione.—To a stirred suspension of 10 g. (0.059 mole) of decalin-2,7-diol in 30 ml. of water cooled to 0° was added a mixture of 8.0 g. (0.08 mole) of chromic anhydride, 6.5 ml. of concentrated sulfuric acid and 40 ml. of water over a period of 2 hours. The temperature was maintained at 0° during the addition. After standing overnight at room temperature the mixture was extracted with ether in a continuous liquid–liquid extraction apparatus. The ether solution was neutralized with solid potassium carbonate and dried over magnesium sulfate. Removal of the solvent left a pale yellow oil which solidified readily. Removal of an oily fraction with a porous plate and then sublimation gave 6.5 g. (43%) of colorless crystals, m.p. 62–64°.

Anal. Calcd. for $C_{10}H_{16}O_2$: C, 72.29; H, 8.44. Found: C, 72.52; H, 8.69.

(2) Melting points were taken on a Fisher–Johns apparatus and are uncorrected.

(3) S. N. Chakravarti and V. Pasupati, *J. Chem. Soc.*, 1859 (1937).

(4) W. S. Johnson, C. D. Gutsche and D. K. Banerjee, THIS JOURNAL, **73**, 5464 (1951).

(5) H. Adkins and H. Billica, *ibid.*, **70**, 697 (1948).

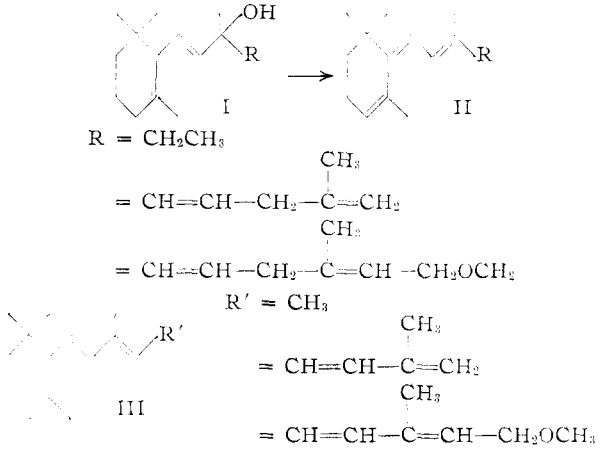
DEPARTMENT OF CHEMISTRY
UNIVERSITY OF WASHINGTON
SEATTLE, WASHINGTON

Conversion of Vitamin A Acetate to Retrovitamin A Acetate

BY R. H. BEUTEL, D. F. HINKLEY AND P. I. POLLAK[1]

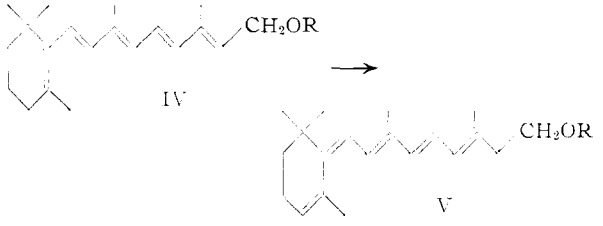
RECEIVED APRIL 23, 1955

In extensive studies of the acid-catalyzed dehydration of β-ionyl alcohols I, Oroshnik and co-workers have observed the preferential formation of conjugated 2,2,6-trimethylcyclohexenylidene derivatives II rather than the formation of the isomeric 2,2,6-cyclohexenyl structures III.[2] In general, substituted cyclohexenes with an endocyclic double bond seem to be more stable than the isomeric exocyclic cyclohexylidenes.[3] It is probable that Brown's hypothesis must be modified in the particular cases studied by Oroshnik in view of the geometries of II and III. Steric interaction of the three methyl ring substituents in III with the side chain on the one hand, and the requirement of maximum coplanarity in order to minimize the energy of the conjugated π-electron system on the other might well favor the formation of II.[2,4]

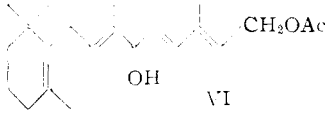


The release of strain in the transition of compounds of series III to those of series II is indicated by the shift of the respective absorption maxima to longer wave lengths, by the concomitant increase in the extinction, and by the appearance of fine structures in the ultraviolet absorption spectra.[2,4]

In the particular case of vitamin A (IV, R = H) it therefore seemed probable that conversion to *retro* vitamin A (V, R = H) should be possible. We have been able to transform vitamin A acetate (IV, R = OAc) to *retro* vitamin A acetate (V, R = OAc) in good yield by treatment with aqueous hydrobromic acid.



The Isler synthesis of vitamin A (IV, R = H) involves the dehydration of the unsaturated alcohol acetate VI.[5]



Oroshnik felt[2] that VI could not be dehydrated to *retro*-vitamin A acetate (V, R = OAc) because of the methylene group adjacent to the cyclohexene ring. We conclude from our observations, however, that the structure of the precursor VI is of secondary importance, and that only the essentially neutral dehydration conditions employed by the Swiss workers[5] permit the isolation of vitamin A acetate (IV, R = OAc). Under acidic conditions any IV formed from VI or any other precursor structure would be converted to the thermodynamically more stable *retro*-vitamin A acetate (V, R = OAc). We postulate that the prototropic isomeri-

(1) To whom inquiries should be addressed.

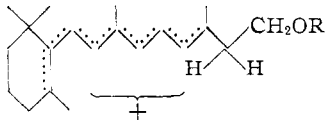
(2) W. Oroshnik, G. Karmas and A. D. Mebane, THIS JOURNAL, **74**, 295, 3807 (1952).

(3) H. C. Brown, J. H. Brewster and H. Shechter, *ibid.*, **76**, 467 (1954).

(4) J. Dale, *Acta Chem. Scand.*, **8**, 1249 (1954).

(5) O. Isler, W. Huber, A. Ronco and M. Kofler, *Helv. Chim. Acta*, **30**, 1911 (1947).

zation of IV to V proceeds through the conjugate acid Va. This intermediate seems reasonable in



Va

view of the well-defined proton acceptor tendencies of polyenes.[6]

Experimental

retro-**Vitamin A Acetate (V, R = OAc).**—Crystalline *trans*-vitamin A acetate (5.00 g.), m.p. 55–58°, λ_{max} 325 mμ, ϵ_{max} 51,000, was dissolved in 50 ml. of methylene chloride. The solution was mixed with 5 ml. of ice-cold concentrated hydrobromic acid. The mixture was shaken in a separatory funnel at 0° for 30 seconds and the layers permitted to separate. The organic layer was washed with 5% aqueous bicarbonate and water. The solvent was removed *in vacuo* at 20°. The resulting red-yellow oil, 4.85 g., had the typical absorption spectrum of *trans-retro*-vitamin A acetate,[2] $\lambda\lambda_{max}$ 333, **348**, 367 mμ, ϵ_{max} 56,800.

A petroleum ether solution of this oil (1.00 g. in 15 ml.) was chromatographed on a 2 × 25 cm. column of carefully neutralized alumina (60–200 mesh). On elution with ether–petroleum ether (1:9) a single band was obtained. A small red band remained essentially stationary at the top of the column. Evaporation of the solvent afforded 0.92 g. of a yellow oil which had the same spectrum as above.

Anal. Calcd. for $C_{22}H_{32}O_2$: C, 80.43; H, 9.82. Found: C, 80.57; H, 9.74; sapn. equiv., 320, 318.

(6) A. Wassermann, *J. Chem. Soc.*, 4329 (1954).

RESEARCH & DEVELOPMENT DEPARTMENT
CHEMICAL DIVISION, MERCK & CO., INC.
RAHWAY, N. J.

Purification and Amino Acid Composition of Melanophore-expanding Hormone from Hog Pituitary Gland[1]

BY B. J. BENFEY AND J. L. PURVIS

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Raben, *et al.*,[2] in 1952, reported that the melanophore hormone, after purification by adsorption on oxycellulose, can be separated from ACTH by partition between certain organic acids and butanol. However, a detailed description of the procedure does not appear to have been published. Other workers,[3,4] more recently, have prepared highly potent ACTH preparations which retained a high degree of melanophore-expanding activity. The presence of the latter property in the purified product led these workers to conclude that the melanophore activity is a property of ACTH. The preparation from hog pituitary of a highly purified, if not pure, melanophore-expanding hormone, free from ACTH, recently has been reported by Lerner and Lee.[5]

The present authors with the aid of countercurrent distribution between 0.5 trichloroacetic acid and secondary butanol, have obtained a highly purified melanophore preparation free from ACTH activity. We have isolated also, from the same starting material in the same countercurrent run, an ACTH preparation which behaves like the unhydrolyzed corticotropin of Kuehl, *et al.*,[6] toward two solvent systems, namely, 0.5% trichloroacetic acid–secondary butyl alcohol and 0.1% trichloroacetic acid–secondary butyl alcohol. Our melanophore preparation behaves like the corticotropin-B (pepsin-hydrolyzed corticotropin-A) of Kuehl, *et al.*,[6] toward the 0.5% trichloroacetic acid–secondary butyl alcohol system. It would appear that, following the hydrolytic treatment used by Kuehl, *et al.*,[6] the partition ratios of our hog melanophore hormone and Kuehl's corticotropin-B are nearly the same for this solvent system, thus possibly accounting for the failure of these workers to separate the two materials by countercurrent distribution between 0.5% trichloroacetic acid and secondary butyl alcohol. The biological potencies of our preparations are given in Table I.

TABLE I

Partition ratio (K)	Melanophore activity (I.U./mg.)*	ACTH activity[7] (I.U./mg.)
0.64	3000	Less than 0.1
7.0	20	14–15

* This is 1500 times as potent as the International Standard Posterior Pituitary Powder (beef), as assayed in the intact frog. The potency of the purified product was between 400–500 times that of the initial hog posterior pituitary powder.

The extraction and purification of melanophore hormone from acetone-dried posterior pituitary powder (hog)[8] was achieved by extraction with acetic acid and adsorption on oxycellulose according to the method of Payne, *et al.*[9] Countercurrent distribution between 0.5% trichloroacetic acid and secondary butyl alcohol was carried out in an all-glass Craig apparatus,[10] using 100 ml. of each phase per tube with 180 transfers. The contents of the tubes, numbers 69 to 71, containing highest melanophore potency (K = 0.64) were concentrated *in vacuo* under nitrogen to a volume of about one ml. The concentrate was transferred to a 100-ml. cylinder with the aid of 4 ml. of methanol and the solute was precipitated by the addition of 85 ml. of ethyl acetate. A portion of the precipitate (14.6 mg.) was put through a 24-transfer countercurrent distribution in a small Craig machine using 0.5% trichloroacetic acid and secondary butyl alcohol as the solvent system. Countercurrent distribution was the only criterion of purity applied. The results of the spectrophotometric analysis are represented in Fig. 1.

(1) This work was supported in part by grants from the Hutchison Fund of McGill University and from Canada Packers Ltd., of Toronto (J. L. P.).

(2) M. S. Raben, I. N. Rosenberg and E. B. Astwood, *Federation Proc.*, **11**, 126 (1952).

(3) N. G. Brink, G. E. Boxer, V. C. Jelinek, F. A. Kuehl, Jr., J. W. Richter and K. Folkers, THIS JOURNAL, **75**, 1960 (1953).

(4) P. H. Bell, *ibid.*, **76**, 5565 (1954).

(5) A. B. Lerner and T. H. Lee, *ibid.*, **77**, 1066 (1955)

(6) F. A. Kuehl, Jr., M. A. P. Meisinger, N. G. Brink and K. Folkers, *ibid.*, **75**, 1955 (1953).

(7) The authors are indebted to M. Saffran and A. V. Schally for carrying out the ACTH assays according to their *in vitro* method (*Endocrinology* in press, 1955). Before assay the preparations were treated with hydrogen sulfide as a precaution against possible loss of activity.[6]

(8) The authors acknowledge the generous gift from Nordic Biochemicals Ltd., Montreal, of acetone-dried hog posterior pituitary powder.

(9) R. W. Payne, M. S. Raben and E. B. Astwood, *J. Biol. Chem.*, **187**, 719 (1950). E. B. Astwood, M. S. Raben, R. W. Payne and A. B. Grady, THIS JOURNAL, **73**, 2969 (1951).

(10) The authors are grateful to Ayerst, McKenna and Harrison, Ltd., Montreal, for making available their countercurrent machines.