solution, when acidified, yielded 0.25 g. of IV. *Anal.* Calcd. for $C_{18}H_{15}N_5O_2$: C, 64.9; H, 4.5; N, 21.0. Found: C, 64.5; H, 4.4; N, 21.1. The cooled alcoholic liquor from A yielded 4.1 g. of starting material II, m.p. 215–220°.

The half of the material, B, which was refluxed longer, yielded the same products as described in the literature,[2] *i.e.*, I and some alcohol-insoluble yellow material. The insoluble yellow product IX (needles), 0.26 g., m.p. >400° (darkens > 360°), was washed with hot alcohol and submitted for analysis without being recrystallized, since no solvent could be found for the material. *Anal.* Calcd. for $C_{27}H_{18}N_6O_3$, IX: C, 68.4; H, 3.8; N, 17.7. Found: C, 68.5; H, 4.0; N, 17.7. No IV was isolated. An attempt was made to acylate the insoluble yellow material IX. It failed to dissolve in boiling 50% acetic anhydride–pyridine solution.

1-Phenyl-3-*p*-toluidino-5-pyrazolone (X).—A solution of 20 g. of II in 50 g. of *p*-toluidine, after being refluxed two hours, was diluted with 100 ml. of chloroform and cooled. The product was collected, washed well with chloroform, and recrystallized twice from 300-ml. portions of ethanol. Again a small yield (1.0 g.) of insoluble yellow needles (IX) was obtained, m.p >400°. *Anal.* Found: C, 68.0; H, 3.9; N, 17.0. The yield of X was 7.0 g., 23%, m.p. 220–223°. *Anal.* Calcd. for $C_{16}H_{15}N_3O$: N, 15.9. Found: N, 15.8.

1-Methyl-3-anilino-5-pyrazolone[7] (XI).—A solution of 1.0 g. of 1-methyl-3-amino-5-pyrazolone[5] in 3.0 ml. of aniline was refluxed 1.5 hours. The cooled solution yielded 0.4 g. of crude material, which was washed well with methanol. When this was treated with 20 ml. of boiling acetonitrile, 0.1 g. failed to dissolve. This material turns blue in the coupling test and is probably a mixture of compounds of types IV and IX. The cooled acetonitrile yielded 0.2 g., 14%, of XI, m.p. 220–222°. *Anal.* Calcd. for $C_{10}H_{11}N_3O$: C, 63.5; H, 5.8; N, 22.2. Found: C, 63.9; H, 5.5; N, 22.1.

The Reaction of 3,3′-Imino-bis-(1-phenyl-5-pyrazolone) (IV) with Aniline.—One gram of IV was suspended in 5 ml. of refluxing aniline for two hours. The material failed to dissolve and 0.7 g. was recovered from the cooled mixture. No other workable product was found.

(7) A. Weissberger and H. D. Porter, THIS JOURNAL, **65**, 732 (1943).

ROCHESTER, N. Y.

[CONTRIBUTION FROM THE RESEARCH LABORATORIES, CHEMICAL DIVISION, MERCK & CO., INC.]

Vitamin B_{12}. XXIII. Resolution of DL-1-Amino-2-propanol

BY ROBERT L. CLARK, WILLIAM H. JONES, WILLIAM J. RAICH AND KARL FOLKERS

RECEIVED FEBRUARY 10, 1954

DL-1-Amino-2-propanol was resolved into $D_g(-)$-1-amino-2-propanol and $L_g(+)$-1-amino-2-propanol by the following sequence of compounds and steps: DL-1-benzylamino-2-propanol, DL-2-(1-benzylaminopropyl)-*p*-nitrobenzoate and resolution of L(+)-tartrates, saponification to D(−)- and L(+)-1-benzylamino-2-propanol, hydrogenation to the D- and L-1-amino-2-propanols.

The "ninhydrin-reacting" substance was first recognized[1] in an acid hydrolysate of vitamin B_{12}. This substance was detected and studied by paper strip techniques, and it was indistinguishable from 2-amino-1-propanol.[2] The identification of the substance with 2-amino-1-propanol was invalidated when it was oxidized with permanganate and did not give alanine.[3]

The structure of this substance reacting with ninhydrin was established[4] as 1-amino-2-propanol (I) by isolation of a dibenzoate of the amino alcohol and its characterization and degradation. Structure I was confirmed by synthesis.[4] This synthetic route was chosen because it would establish configuration; it showed that the substance is D_g-1-amino-2-propanol. D_g-Lactic acid was converted to D_g-1-amino-2-propanol by esterification, conversion to the amide and reduction with lithium aluminum hydride.

The first determinations[5] of the amount of the aminopropanol liberated in the hydrolysis of vitamin B_{12} indicated two moles per molecule of vitamin B_{12}. The analytical method was based upon a quantitative determination of ethanolamine. Later determinations[6] of the liberated D_g-1-amino-2-propanol have led to the statement that there is only one molecule of the substance per molecule of vitamin B_{12}. This conclusion is based upon the results of two methods: (a) quantitative measurement of the ninhydrin reaction product by comparison of absorption with controls; (b) differential determination of ammonia and "total ammonia" after formation of ammonia from 1-amino-2-propanol by periodate oxidation.

The most recent report[7] on the amount of liberated 1-amino-2-propanol confirmed the data of Cooley, *et al.*,[6] for the same hydrolytic conditions, but stronger acid hydrolysis in some experiments gave results approximating two moles of 1-amino-2-propanol. Nevertheless, these investigators[7] favored the conclusion[6] on one mole and gave no explanation of the high "anomalous" results.

The value of the synthesis[4] of D_g-1-amino-2-propanol was confirmation of structure and configuration, but not for convenient preparation. DL-1-Amino-2-propanol is available commercially; therefore, it was desirable to study methods for its resolution. No procedures for the resolution of DL-1-amino-2-propanol could be found in the literature.

Although several salts of the aminopropanol were examined for possible direct resolution, none of them crystallized. A derivative was prepared by which resolution was accomplished with salts of $L(^+)$-tartaric acid.

DL-1-Amino-2-propanol reacted with benzaldehyde and the Schiff base was hydrogenated with

(1) B. Ellis, V. Petrow and G. F. Snook, *J. Pharm. Pharmacol.*, 1, 60 (1949).

(2) B. Ellis, V. Petrow and G. F. Snook, *ibid.*, 1, 735 (1949); 1, 950 (1949).

(3) G. Cooley, B. Ellis and V. Petrow, *ibid.*, **2**, 128 (1950).

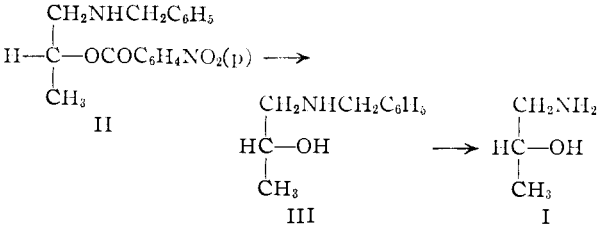
(4) D. E. Wolf, W. H. Jones, J. Valiant and K. Folkers, THIS JOURNAL, **72**, 2820 (1950).

(5) E. Chargaff, C. Levine, C. Green and J. Kream, *Experientia*, **6**, 229 (1950).

(6) G. Cooley, M. T. Davies, B. Ellis, V. Petrow and B. Sturgeon *J. Pharm. Pharmacol.*, **4**, 257 (1953).

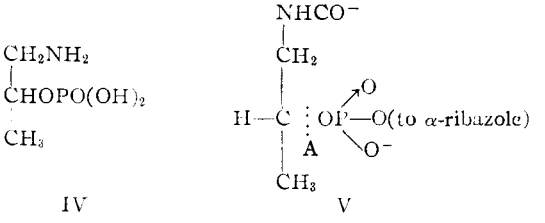
(7) J. B. Armitage, J. R. Cannon, A. W. Johnson, L. F. J. Parker, E. L. Smith, W. H. Stafford and A. R. Todd, *J. Chem. Soc.*, 3849 (1953).

Raney nickel catalyst to give over 82% of oily DL-1-benzylamino-2-propanol. The next reaction, which was with *p*-nitrobenzoyl chloride in chloroform and the base hydrochloride, gave a 44% yield of crystalline DL-2-(1-benzylaminopropyl)-*p*-nitrobenzoate as the hydrochloride. The L(+)-tartrate of the desired "natural" derivative of the D-isomer (II) was the least soluble in acetone and was obtained in a 71% yield. The L(+)-tartrate of the derivative of the L-isomer was obtained crystalline from the mother liquor. Alkaline hydrolysis of the esters gave the D-(III) and L-isomers of 1-benzylamino-2-propanol, which were hydrogenated over palladium catalysts to $D_g(-)$-1-amino-2-propanol (I) and $L_g(+)$-1-amino-2-propanol.



DL-1-Benzylamino-2-propanol has been studied by others.[8,9] Since esters containing primary or secondary amino groups rearrange[10] into the corresponding amides the N-benzyl derivative was used because the ester containing a secondary amino group would be expected to rearrange less readily than the corresponding compound containing the primary amino group (*i.e.*, 2-(1-aminopropyl)-*p*-nitrobenzoate).

The preparation of DL-1-amino-2-propanol O-phosphate as a crystalline free acid IV has recently been described.[6]



Acid hydrolysis of this O-phosphate will not liberate the 1-amino-2-propanol when the method is used which does liberate the amino alcohol from vitamin B_{12}. This O-phosphate IV has been contemplated[6,11] as a moiety of vitamin B_{12}. While this acid stability of the phosphate may be understandable,[6] it is noted that not one of four different groups of investigators ever reported a trace of a "second ninhydrin-reacting" spot (*i.e.*, the phosphate) by sensitive paper chromatography. Such negative results on the hydrolytic liberation of this phosphate necessitate almost quantitative preferential cleavage of linkage A in structure V over the amide linkage and the phosphate linkage to α-ribazole and perhaps another position in vitamin B_{12}.

(8) A. Uedinck, *Ber.*, **32**, 969 (1899).

(9) P. Staub, *Helv. Chim. Acta*, **5**, 888 (1922).

(10) A. C. Cope and E. M. Hancock, THIS JOURNAL, **66**, 1448 (1944).

(11) J. G. Buchanan, A. W. Johnson, J. A. Mills and A. R. Todd, *Chemistry and Industry*, 426 (1950).

Experimental

DL-1-Benzylamino-2-propanol.—To a solution of 300 g. (4 moles) of 1-amino-2-propanol (Eastman Kodak Co.) in 400 ml. of ethanol, 424 g. (4 moles) of benzaldehyde was added in small portions with shaking. After about one-half of the benzaldehyde had been added, 250 ml. of ethanol was added to cool the reaction mixture. After all the benzaldehyde had been added, the temperature had risen to 50–60°. The mixture was cooled to room temperature, and the total volume of ethanol in the mixture was increased to 1200 ml. Hydrogenation was carried out at a pressure of 1800 lb./sq. in. and at room temperature with 6 g. of Raney nickel catalyst. It was complete after 12 hours. The catalyst was removed by filtration and the solvent removed *in vacuo*. The yellow oily residue was distilled through a Vigreux column at 3 mm.; the fraction boiling at 120–130° was collected; $n^{25}D$ 1.5268. The yield of this fraction was 541 g. or 82%. Another 25 g. of product was obtained by combining the forerun with the residue and redistilling.

DL-2-(1-Benzylaminopropyl)-*p*-nitrobenzoate Hydrochloride.—A solution of 331 g. (2 moles) of 1-benzylamino-2-propanol in 600 ml. of dry chloroform was saturated at 0° with dry hydrogen chloride, and added to a solution of 376 g. (2.02 moles) of *p*-nitrobenzoyl chloride in 600 ml. of dry chloroform. The volume of the mixture was brought to 2300 ml. with dry chloroform and heated on the steam-bath for 70 hours. The chloroform was removed *in vacuo*, and the light yellow residue was triturated with 1 liter of ether. After cooling for two hours, the mixture was filtered, and the residue was washed with ether. The crude material on recrystallization from 5 liters of methanol, gave 270 g. of crystalline product, m.p. 222–223°. Concentration of the mother liquors gave an additional 36 g. of material, m.p. 217–221°. The yield of first and second crop material was 43.5%.

Anal. Calcd. for $C_{17}H_{18}O_4N_2 \cdot HCl$: C, 58.20; H, 5.46; N, 7.99. Found: C, 58.06; H, 5.25; N, 8.10.

D-(−)- and L(+)-2-(1-Benzylaminopropyl)-*p*-nitrobenzoate L(+)-Acid Tartrate.—A suspension of 20 g. of 2-(1-benzylaminopropyl)-*p*-nitrobenzoate hydrochloride in 250 ml. of 2.5 *N* sodium hydroxide was rapidly stirred mechanically with 400 ml. of ether until the solid had disappeared. The aqueous layer was separated and extracted with 120 ml. of ether. The combined ether extract was washed with three 80-ml. portions of water. After drying with anhydrous magnesium sulfate, the ether solution of the amine was added slowly with stirring to a solution of 9 g. of L(+)-tartaric acid in 30 ml. of ethanol and 500 ml. of ether. The sticky precipitate and milky suspension partially crystallized after standing 16 hours. The mixture was filtered and the material was crystallized from about 2 liters of acetone.

The recrystallized material (13.5 g.) was added to 250 ml. of acetone and the mixture was refluxed for one hour and then filtered. This procedure was repeated until the fine crystals remaining undissolved showed the specific rotation $[\alpha]^{25}D$ −30° (*c*, 1 in methanol). The combined acetone filtrate was cooled and the crystalline material was treated in a similar manner. The total yield of D(−)-2(1-benzylaminopropyl)-*p*-nitrobenzoate L(+)-acid tartrate hydrate was 9.9 g. (71%), m.p. 135–137°.

Anal. Calcd. for $C_{21}H_{24}O_{10}N_2 \cdot H_2O$: C, 52.28; H, 5.43; N, 5.81. Found: C, 52.41; H, 5.21; N, 5.57.

The acetone filtrates were combined and concentrated to a yellow sirup. On addition of ether, crystals formed which were removed by filtration. Rapid trituration of this crystalline material with approximately "10 volumes" of cold methanol gave a granular crystalline product; $[\alpha]^{25}D$ +50° (*c* 1 in methanol); m.p. 145–146°. The L-(+)-2(1-benzylaminopropyl)-*p*-nitrobenzoate L(+)-acid tartrate was recrystallized from methanol–ether, m.p. 145–148°.

Anal. Calcd. for $C_{21}H_{24}O_{10}N_2$: C, 54.31; H, 5.21; N, 6.03. Found: C, 54.32; H, 5.21; N, 5.89.

D(−)- and L(+)-1-Benzylamino-2-propanol Hydrochloride.—To a solution of 24 g. of D(−)-2-(1-benzylaminopropyl)-*p*-nitrobenzoate acid tartrate hydrate in 200 ml. of warm methanol, was added a solution of 45 g. of potassium hydroxide in 200 ml. of methanol. An immmediate precipitate of potassium tartrate separated. This precipitate was removed by filtration, heated with 100 ml. of methanol and the mixture filtered. After the combined filtrate was

heated under reflux 17 hours, the solvent was removed *in vacuo*. As soon as solid began to separate, water was added to maintain a homogeneous solution. After the methanol was removed, the aqueous solution was extracted with five 80-ml. portions of ether. The combined ether solution was washed, dried, and the solvent removed by distillation. The remaining oil was dissolved in 75–100 ml. of dry ether and dry hydrogen chloride added. The resulting precipitate was collected and crystallized from 30 ml. of ethanol by the slow addition of 200 ml. of ether. The yield of D(−)-1-benzylamino-2-propanol hydrochloride was 7.1 g.; m.p. 124–125°; $[\alpha]^{25}D$ −28.4° (*c* 1 in methanol).

Anal. Calcd. for $C_{10}H_{15}ON\cdot HCl$: C, 59.54; H, 8.00; N, 6.95. Found: C, 59.60; H, 7.99; N, 6.88.

L(+)-1-Benzylamino-2-propanol hydrochloride was obtained in the same manner as the D-isomer; m.p. 125–126°; $[\alpha]^{25}D$ +28.4° (*c* 1 in methanol).

Anal. Calcd. for $C_{10}H_{15}ON\cdot HCl$: C, 59.54; H, 8.00; N, 6.95. Found: C, 59.27; H, 7.91; N, 6.69.

D(−)- and L(+)-1-Amino-2-propanol Hydrochloride.—A solution of 7.0 g. of D(−)-1-benzylamino-2-propanol hydrochloride in 100 ml. of methanol was hydrogenated at a pressure of 40 lb./sq. in. over 1.5 g. of 5% palladium on Darco. After 24 to 30 hours, the theoretical amount of hydrogen was absorbed. The catalyst was removed by filtration and the solvents removed *in vacuo*. The residue was crystallized from 20 ml. of ethanol by the addition of 100 ml. of ether. After one more crystallization, the hygroscopic D(−)-1-amino-2-propanol hydrochloride melted at 94–95°; $[\alpha]^{25}D$ −31.5° (*c* 1 in methanol). The yield was 3 g.

Anal. Calcd. for $C_3H_9ON\cdot HCl$: C, 32.39; H, 9.03; N, 12.59. Found: C, 32.32; H, 8.74; N, 12.40.

L(+)-1-Amino-2-propanol hydrochloride was prepared in the same manner; $[\alpha]^{25}D$ +35° (*c* 1 in methanol).

Anal. Calcd. for $C_3H_9ON\cdot HCl$: C, 32.29; H, 9.03; N, 12.59. Found: C, 32.22; H, 8.80; N, 11.84.

RAHWAY, NEW JERSEY

[CONTRIBUTION FROM THE KEDZIE CHEMICAL LABORATORY, MICHIGAN STATE COLLEGE]

The Origin of the Methyl Group of Nicotine through Transmethylation[1]

BY LOVELL J. DEWEY, RICHARD U. BYERRUM AND CHARLES D. BALL

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A study in which methionine, doubly labeled in the methyl group with C^{14} and deuterium, was administered to intact tobacco plants has established that the methyl group of methionine can give rise to the methyl group of nicotine through transmethylation.

In a previous publication it was shown that the methyl carbon of methionine can give rise to the methyl carbon of nicotine.[2] It was also shown that the methyl carbon of methionine was incorporated at a much faster rate than the carbon of formate into the methyl group of nicotine. This was taken as an indication that the methionine methyl group was not oxidized to formate before its incorporation into nicotine. One mechanism proposed for the incorporation of methionine methyl groups into nicotine was that the methyl group was transferred as a unit. Transmethylation, the intact transfer of methyl groups, has previously been established as a reaction in animal metabolism,[3] and recently it was reported that the methoxyl groups of the plant cell wall constituent, lignin, can arise from the direct transfer of methyl groups from methionine.[4,5] The latter reaction involves the transfer of a methyl group from sulfur ultimately to oxygen. However, direct transfer of methyl groups from methionine to give the methyl group of nicotine remained to be demonstrated.

In the present study we have administered DL-methionine, doubly labeled in the methyl group with C^{14} and deuterium to intact *Nicotiana rustica* and have succeeded in showing that the methionine methyl group may be transferred as a unit to form the methyl group of nicotine.

Experimental

Synthesis of Labeled Compounds.—The labeled methionine used in these experiments was synthesized from methyl iodide and DL-homocystine essentially according to the method of du Vigneaud, Dyer and Harmon.[6] In the case of the DL-methionine labeled with deuterium in the methyl group, deuterated methyl iodide was used. The DL-C^{14}-methylmethionine was synthesized from C^{14}-methyl iodide. Both labeled samples of methyl iodide were purchased from Tracerlab, Inc., Boston. The doubly labeled methionine was then obtained by mixing the C^{14}-labeled methionine with the deuterated methionine in the ratio of 10 to 90% (by weight), respectively.

Preparation of Tobacco Plants.—*Nicotiana rustica*, var. *humilis*, a high nicotine strain, was used in these studies. The plants were grown for two months in flats containing vermiculite,[7] which provided a base for the growing plants but gave no nutrients. The plants were watered twice a week with a nutrient solution.[8]

At the end of two months when the plants were about six inches high they were removed from the flats and the roots were freed of vermiculite by soaking and washing in tap water. The roots were then immersed in a 0.1% solution of detergent germicide[9] for 0.5 hour, with occasional agitation, to reduce the bacterial population. After rinsing under tap water, the plants were placed in 125-ml. erlenmeyer flasks containing 50 ml. of an inorganic nutrient medium prepared by diluting, with two parts of water, one part of a stock solution which had the following composition: water, 1 l.; calcium nitrate, 1 g.; potassium chloride, 250 mg.; potassium dihydrogen phosphate, 250 mg.; magnesium sulfate, 250 mg.; ammonium sulfate, 250 mg.; ferric chloride, 2 mg. The weights are of the anhydrous salts. Six drops of 1% germicide solution were added to each flask.

During the administration of the labeled methionine the plants were grown in a hood. Two 36-inch, 30-watt fluorescent tubes and a 100-watt incandescent bulb were placed

(1) This paper is based upon work performed under contract No. AT(11-1)-161 with the Atomic Energy Commission.

(2) S. A. Brown and R. U. Byerrum, THIS JOURNAL, **74**, 1523 (1952).

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(4) R. U. Byerrum, L. J. Dewey and C. D. Ball, *Federation Proc.*, **12**, 186 (1953).

(5) R. U. Byerrum, J. H. Flokstra, L. J. Dewey and C. D. Ball, *J. Biol. Chem.*, in press.

(6) V. du Vigneaud, H. M. Dyer and J. Harmon, *J. Biol. Chem.*, **101**, 719 (1933).

(7) Vermiculite is a commercially available heat-expanded mica.

(8) This nutrient solution was prepared from plant nutrient tablets obtained from Cargille Scientific, Inc., New York, New York.

(9) Wyandotte detergent germicide No. 1528 obtained from the Wyandotte Chemicals Corporation, Wyandotte, Michigan.