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

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

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

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).

(3) E. B. Keller, J. R. Rachele and V. du Vigneaud, *J. Biol. Chem.*, **177**, 733 (1949).

(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.

TABLE I

DEUTERIUM AND C^{14} IN THE FED METHIONINE AND IN THE NICOTINE DIPICRATE

Compound	Excess D, %		C^{14} content in c.p.m./mmole		Ratio of D in methyl group to C^{14} in methyl group	% Nicotine dipicrate ratio of methionine ratio
	Compound	Methyl group	Compound	Methyl group		
	(A)	(B)	(C)	(D)	(B/D)	
DL-Doubly-labeled methionine	24.5	89.8	2.07×10^5	2.07×10^5	4.34×10^{-4}	..
Nicotine dipicrate	0.243	1.62	3.96×10^3	3.96×10^3	4.09×10^{-4}	94

about 14 inches above the tops of the plants and the light intensity at the level of the upper leaves was found to be 200 to 250 foot-candles. The lights were left on 12 hours out of 24 during the entire administration period. Additional nutrient solution was added to the flasks to keep the volume constant. Initial wilting of some of the plants was encountered, but this was overcome by bubbling oxygen through the nutrient solution of each plant for two minutes twice a day.

Administration of Doubly Labeled Methionine.—Previously it was shown that tobacco plants absorb methionine from a nutrient medium through the root systems.[2] Sixty tobacco plants were prepared for the hydroponic administration of the labeled methionine as described above. They were allowed to grow in the nutrient solution for nine days to establish the root systems. On the tenth day three plants were discarded because of wilting, and then 2 mg. of doubly labeled methionine was added to the nutrient solutions in the flasks containing the remaining plants. During the rest of the growing period the plants grew as was evidenced by increase in stem length, production of leaves and growth of roots. Four and nine days later 2 mg. more of labeled methionine was added to the flasks making a total of 6 mg. of labeled methionine fed to each plant. The labeled methionine had an atom per cent. excess D of 24.5 and the total count received by each plant was 8×10^8 c.p.m. Thirteen days after the first methionine had been administered the plants were removed from the flasks and the roots were rinsed with distilled water. The plants then were cut into small pieces with scissors and immediately dried as rapidly as possible under heat lamps. The temperature of the drying plants reached about 80° in 15 minutes.

Isolation and Purification of Nicotine.—The nicotine was isolated from the dried tobacco plants as the dipicrate, which was purified according to the method described in a previous paper.[2]

Since it was previously shown[2] that within experimental error all of the radioactivity in the nicotine isolated from tobacco plants which had been fed C^{14}-methyl methionine was located in the methyl group, the nicotine was not demethylated in the present study but was used for further experiments as such.

Determination of the Isotopes.—All counts were made in a windowless flow counter (Tracerlab, Inc.) using a Nuclear Instrument and Chemical Corp. scaler. The gas used in the flow counter was a mixture of helium (99.05%) and isobutane (0.95%) and was purchased from The Matheson Co., Inc., East Rutherford, New Jersey. The over-all efficiency of the counter was 19% as determined using a National Bureau of Standards sodium bicarbonate sample.

For the determination of radioactivity the nicotine dipicrate and methionine were burned in a combustion train at 700°. The combustion tube used in the train was packed with the "combination filling" of CuO and platinized asbestos described by Niederl and Niederl.[10] The CO_2 of combustion was precipitated as barium carbonate from a barium hydroxide solution and was counted in aluminum dishes. Since only a comparison of activities was desired, 40 mg. of barium carbonate was counted in each case and the count was not corrected for self-absorption.

A trap immersed in a solid carbon dioxide–methyl cellosolve bath was placed in the combustion train before the barium hydroxide solution to freeze out the water of combustion. The water was purified and its deuterium content determined by the gradient tube method described by Linderstrom-Lang, et al.[11] We are indebted to Dr. David

(10) J. B. Niederl and V. Niederl, "Micromethods of Quantitative Organic Analysis," 2nd ed., John Wiley and Sons, Inc., New York, N. Y., 1942, p. 108.

(11) K. Linderstrom-Lang, O. Jacobsen and G. Johansen, *Compt. rend. trav. lab. Carlsberg*, **23**, 17 (1938).

Rittenberg and Laura Ponticorvo for the determination of the deuterium content of the nicotine dipicrate.

Exchange Study.—Although the possibility of an uncatalyzed exchange of methyl groups between methionine and nicotine during the feeding period and isolation of the nicotine from the plants seemed remote, an experiment was set up to check this possibility. Seventy mg. of nicotine and 50 mg. of C^{14}-methylmethionine were dissolved in 250 ml. of a 0.05 M phosphate buffer, pH 6.6. After this mixture had stood for eight days at room temperature, the nicotine was separated by an azeotropic distillation with water and isolated from the distillate as the dipicrate. The dipicrate was counted in an aluminum dish in the flow counter and no significant count over background was obtained. This experiment indicates that under conditions comparable to the conditions of the feeding experiments there was no detectable, uncatalyzed interchange of the methyl groups between nicotine and methionine.

Results and Discussion

The atom per cent. excess deuterium and the radioactivity of the fed methionine and the isolated nicotine dipicrate are presented in columns A and C of Table I. In column B is shown the % excess D in the methyl groups of the labeled methionine and dipicrate. The data in column B are values calculated on the assumption that all of the excess deuterium is located in the methyl groups of the respective compounds. In the case of the methionine the compound was synthesized from deuterated methyl iodide and homocystine and the methyl group is the only position where the excess deuterium may be located. In the case of the nicotine dipicrate it has been shown that within experimental error all of the C^{14} in the nicotine isolated from plants fed C^{14}-methylmethionine was located in the methyl group of the nicotine.[2] The above assumption, therefore, seems justified in this study. By similar argument the radioactivity of the methyl groups of the two compounds is assumed to be the same as the radioactivity of the respective compounds. The radioactivity (counts per minute (c.p.m.) per mmole) of the compounds and methyl groups are given in columns C and D, respectively.

The ratios of the deuterium to the radioactivity in the methyl group of the nicotine dipicrate and the methionine are shown in the column subtitled (B/D) in Table I. In the last column it is shown that the ratio of deuterium to C^{14} in the methyl group of the nicotine dipicrate is 94% of the ratio of deuterium to C^{14} in the methyl group of the methionine. This percentage represents the per cent. of the deuterium, originally present in the methionine methyl group, which was transferred with the methyl carbon. If transmethylation occurs then of course 100% of the deuterium would remain in the methyl group. However, if the methyl group of methionine is oxidized to the state of formaldehyde and later reduced, only 67% of the original deuterium can remain with the methyl carbon. Since 94% of the deuterium remained with the methyl carbon during its transfer from methionine in the

present study, it is concluded that a large proportion of the methyl groups, which are transferred from methionine, are transferred through a transmethylation process. This work, along with the finding[4,5] that the methoxyl groups of lignin can arise from the direct transfer of methyl groups from methionine, indicates the generality of the transmethylation reaction in higher plants.

The question as to whether this transmethylation actually represents a net biosynthesis of nicotine still remains unproved. Since it has been shown that nicotine is synthesized mainly in the roots,[12] one approach to this question would be to ascertain whether or not a net synthesis of nicotine could be obtained by incubating root enzyme preparations with methionine and nornicotine. Such a study is in progress at the present time in this Laboratory, and the results will be forthcoming at a later date.

(12) R. F. Dawson, *Am. J. Botany*, **29**, 66 (1942).

EAST LANSING, MICHIGAN

Hypotensive Methoxyisoquinolines

BY GORDON N. WALKER

RECEIVED MARCH 8, 1954

Dehydronorcoralydine iodide (II) was synthesized. Hydrochlorides of 3-(3',4'-dimethoxyphenyl)-6,7-dimethoxyisoquinoline, 1-methyl-4-(3',4'-dimethoxyphenyl)-6,7-dimethoxyisoquinoline, 1-methyl-4-phenyl-6,7-dimethoxyisoquinoline, 1-methyl-6,7-dimethoxyisoquinoline and 5-methyl-2,3,10,11-tetramethoxybenzo[a]phenanthridine (IX) were prepared by phosphorus oxychloride cyclization of appropriate amides, dehydrogenation and treatment with hydrogen chloride. These compounds elicited a lowering of blood pressure in normal dogs. Certain modified procedures for synthesis of the compounds and their precursors are described.

It has been reported[1] that the alkaloid palmatine (I) effects a lowering of blood pressure and at the same time paralyzes the central nervous system and respiratory center, upon intravenous administration in mammals. Two related monophenolic alkaloids, jateorhizine and columbamine,[2] also produce these effects to a lesser extent. These facts prompted an investigation of the chemistry and pharmacology of several related classes of compounds incorporating methoxyl groups and the isoquinoline ring system.

Dehydronorcoralydine iodide (II) which differs from palmatine only in the position of one methoxyl group, was synthesized by the method of Pictet and Chou,[3] consisting of dehydrogenation of norcoralydine with iodine. Norcoralydine was prepared by cyclization of tetrahydropapaverine with formaldehyde in the presence of hydrochloric acid, according to the directions of Craig and Tarbell.[4] This cyclic condensation gave, in our hands, two different forms of norcoralydine, both of which led to the same dehydroiodide when treated with iodine. In the preparation of tetrahydropapaverine, hydrogenation of the carbon–nitrogen double bond and hydrogenolysis of the carbonyl group of 3,4-dihydropapaveraldine were accomplished in one step by the use of palladium–charcoal catalyst in glacial acetic acid at 80°, a method apparently not used heretofore in obtaining tetrahydroisoquinolines.

Compound IV, a 3-arylisoquinoline closely related in structure to dehydronorcoralydine, was obtained by cyclization of the amide IIIa with phosphorus oxychloride, followed by dehydrogenation (palladium). The substituted formamide IIIa was prepared from 3,4,3',4'-tetramethoxydesoxybenzoin by the Leuckart reaction. In contrast to the formamide, the corresponding acetamide IIIb did not cyclize when treated with phosphorus oxychloride under the same conditions, but instead appeared to undergo elimination of acetamide at least in part, with the formation of 3,4,3',4'-tetramethoxystilbene. The difference in behavior of IIIa and IIIb in the presence of phosphorus oxychloride cannot be explained readily.

Two methoxylated 1-methyl-4-phenylisoquinolines, VIa and VIb, were prepared by a series of steps as follows: Reformatsky reaction of 3,4,3',4'-tetramethoxybenzophenone and 3,4-dimethoxybenzophenone with ethyl bromoacetate and hydrogenolysis of the resulting hydroxyesters provided ethyl β,β-diarylpropionates, which were converted quantitatively to the corresponding acid hydrazides by means of anhydrous hydrazine. The corresponding acid azides were prepared from the acid hydrazides by reaction with nitrous acid. The acid azides rearranged smoothly in the presence of acetic acid and acetic anhydride and afforded the N-substituted acetamides Va and Vb, respectively. This procedure also was employed in preparing IIIb from ethyl α,β-di-(3,4-dimethoxyphenyl)-propionate. Cyclization of Va and Vb with phosphorus oxychloride and dehydrogenation (palladium) of the resulting 3,4-dihydroisoquinolines gave VIa and VIb, respectively. A methoxylated tetrahydrobenzophenanthridine VIII was prepared from 1-(3',4'-dimethoxyphenyl)-2-carbethoxy-6,7-dimethoxytetralin by the same series of steps, involving Curtius rearrangement of the corresponding acid azide and cyclization of amide VII. Palladium dehydrogenation of VIII led to elimination of two molecules of hydrogen and the formation of the fully aromatic structure IX, while hydrogenation

(1) J. Biberfeld, *Zeit. exp. Path. Pharm.*, **7**, 569 (1910).

(2) For the structures of these three alkaloids, *cf.* E. Späth and co-workers, *Ber.*, **58**, 1939, 2267 (1925); **59**, 1486 (1926), and earlier references.

(3) A. Pictet and T. Q. Chou, *Ber.*, **49**, 370 (1916).

(4) L. E. Craig and D. S. Tarbell, THIS JOURNAL, **70**, 2783 (1948).