Anal. Calcd. for $C_{12}H_{19}O_5P$: P, 11.3. Found: P, 11.0.

Manometric Determination of Reactivity.—These experiments were carried out in conventional Warburg respirometers, carrying conical vessels of 17–20-ml. capacity with one or two vented side-arms. The phenolic compound under investigation was dissolved in water, neutralized to phenol red if necessary, and pipetted into the main part of the vessel. Sufficient water and $NaHCO_3$ solution were added to achieve a final volume of 2.2 ml. and a concentration of 0.025 *M* $NaHCO_3$. The vessels were gassed on the manometers for 10 minutes with a stream of 5% CO_2 in nitrogen. At the end of this time, solutions of the required strength of DFP in 0.025 *M* $NaHCO_3$ were prepared and added to the side arms which were then stoppered. The manometers were closed off and shaken in a constant temperature-bath at 25° for 10 minutes. After mixing the contents of main vessel and side arm, the rate of CO_2 evolution was followed at suitable intervals in the usual manner.

ARMY CHEMICAL CENTER, MARYLAND

RECEIVED AUGUST 23, 1951

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

The Origin of the Methyl Carbon of Nicotine Formed by *Nicotiana Rustica L.*[1]

BY STEWART A. BROWN[2] AND RICHARD U. BYERRUM

Tracer experiments with C^{14} have established that the methyl carbon of methionine can act as a precursor of the nicotine methyl carbon in intact *Nicotiana rustica* plants. A lesser incorporation of formate carbon into the methyl group of nicotine was observed. It is considered probable that formate is employed by the plant in the synthesis of labile methyl groups, which then undergo transmethylation to nicotine.

Transmethylation has been well established in the animal,[3] but although its existence has been postulated[4] the reaction has not been established by direct experimentation in the higher plant. Barrenscheen and von Vályi-Nagy[5] have obtained evidence, *in vitro*, for its occurrence in wheat germ, and Dawson[6] formerly considered that the origin of nornicotine in the tobacco leaf could best be explained by a transmethylation reaction, but recently he has shown that this reaction is a relatively non-specific N-dealkylation of nicotine. Kirkwood and Marion,[7] after feeding experiments with C^{14}-methyl choline, concluded that the N-methyl groups of the alkaloid hordenine do not arise from the choline–methionine system in barley.

We have administered C^{14}-methyl-labeled methionine, a methyl donor in the animal,[8] to intact plants of *Nicotiana rustica*, and have succeeded in establishing the transfer of the methyl carbon to the methyl group of nicotine. In addition, we have obtained some evidence, through the use of C^{14}-formate, that this transfer does not take place with an intermediate oxidation and reduction.

Experimental

Synthesis of C^{14}-Labeled Compounds.—DL-C^{14}-Methyl methionine was synthesized from C^{14}-methyl iodide (purchased from Tracerlab, Inc., Boston) essentially according to the method of Melville, Rachele and Keller.[9] The DL-S-benzyl homocysteine used in this synthesis was prepared from commercially available DL-homocystine as described by du Vigneaud and Patterson.[10] The C^{14}-formate was purchased as the sodium salt from the Oak Ridge laboratories of the United States Atomic Energy Commission.

Preparation of the Tobacco Plants.—*Nicotiana rustica* var. humilis, a high nicotine strain, was used in these studies. The seeds were planted in flats in the greenhouse, and the seedlings were transplanted after about three weeks into small pots, where they were grown until they had reached a height of at least six inches. During this period, from two to three months, they were occasionally supplemented with commercial plant food mixture as required.

To prepare the plants for hydroponic administration of the radioactive materials, as much as possible of the adhering soil was removed from the roots, first by shaking and then by washing under a stream of tap-water. The roots were then immersed in a 0.01% solution of Wyandotte detergent germicide No. 1528[11] for at least one hour, with occasional agitation. Tests carried out by the Department of Horticulture at this institution have shown that this compound has no deleterious effect on plant growth,[12] and its use was considered advantageous to reduce the bacterial population. Following a brief rinse under tap water, the roots of each plant were placed in 50 ml. of an inorganic nutrient medium in a 125-ml. erlenmeyer flask. This medium was prepared by diluting, with two parts water, one part of a stock solution the composition of which was as follows: water, 1 l.; calcium nitrate, 1 g.; potassium chloride, 250 mg.; magnesium sulfate, 250 mg.; ammonium sulfate, 250 mg.; potassium dihydrogen phosphate, 250 mg.; ferric chloride, 2 mg. The weights are of the anhydrous salt.

During the administration of the isotope it was considered advisable to grow the plants in a hood, because of a possible health hazard from $C^{14}O_2$ liberated through respiration. Two 36-inch, 30-watt fluorescent tubes and a 100-watt incandescent bulb were placed 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 light was left on approximately 12 hours out of 24 while the plants were growing. Distilled water was added as required.

Although Steinberg[13] has shown that methionine can exert a toxic influence on tobacco plants under certain conditions, this appears not to be the case with the much lower concentrations and shorter exposure times employed here.

(1) This paper is based on a thesis presented by Stewart A. Brown in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in the Graduate School of Michigan State College. The work was supported in part by an All College Research Grant and the U. S. Atomic Energy Commission.

(2) Eastman Kodak Fellow, 1950–1951.

(3) (a) V. du Vigneaud, J. P. Chandler, M. Cohn and G. B. Brown, *J. Biol. Chem.*, **134**, 787 (1940); (b) V. du Vigneaud, M. Cohn, J. P. Chandler, J. R. Schenck and S. Simmonds, *ibid.*, **140**, 625 (1941); (c) J. R. Schenck, S. Simmonds, M. Cohn, C. M. Stevens and V. du Vigneaud, *ibid.*, **149**, 355 (1943).

(4) (a) R. Robinson, *J. Roy. Soc. Arts*, **96**, 795 (1948); (b) H. B. Vickery, *Biological Symposia*, **5**, 3 (1941).

(5) H. K. Barrenscheen and T. von Vályi-Nagy, *Z. physiol. Chem.*, **277**, 97 (1942).

(6) (a) R. F. Dawson, *Am. J. Botany*, **32**, 416 (1945); (b) R. F. Dawson, THIS JOURNAL, **73**, 4218 (1951).

(7) S. Kirkwood and L. Marion, *Can. J. Chem.*, **29**, 30 (1951).

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

(9) D. B. Melville, J. R. Rachele and E. B. Keller, *ibid.*, **169**, 419 (1947).

(10) V. du Vigneaud and W. I. Patterson, *ibid.*, **109**, 97 (1935).

(11) This material was obtained from the Wyandotte Chemicals Corp., Wyandotte, Michigan, through the Michigan State College, Department of Horticulture.

(12) E. H. Lucas, private communication.

(13) R. A. Steinberg, *J. Agr. Research*, **78**, 733 (1949).

In the few instances in which the condition of a plant appeared abnormal it was rejected before working up the plant material for nicotine. This procedure was also adhered to in the later runs with formate. With these few exceptions the plants retained a healthy appearance throughout a seven-day period, and most showed a definite increase in size. The roots appeared normal except for a slight darkening toward the end of the period, and exhibited growth as evidenced by the production of root hairs. The latter point may be significant in that nicotine synthesis has been shown to parallel protein formation.[14]

Isolation and Purification of Nicotine.—The tobacco plants were removed from the medium and the roots rinsed with distilled water, the excess being blotted off with cheesecloth. The plants were then cut into small pieces with scissors, pooled, and dried as rapidly as possible under heat lamps. Toward the end of the drying period the temperature was maintained at 100° for about an hour. The dried material was ground finely in a mortar, mixed with about one-tenth of its weight of calcium hydroxide, and steam-distilled. The distillate was concentrated, and the alkaloid purified by two successive azeotropic distillations from alkaline medium into hydrochloric acid as described by Smith.[15] The water was then removed from the distillate *in vacuo*. The residue of nicotine hydrochloride was dissolved in methanol plus a little water, and a saturated methanolic solution of picric acid was added in excess. After standing for a short time the precipitate of nicotine dipicrate was filtered off, washed with methanol, and recrystallized from hot water (m.p. 224–225°, recorded value 224°[16]).

Degradation of Nicotine.—An adaptation of the Herzig and Meyer technique[17,18] for the estimation of methyl- and ethyl-imino groups was employed to obtain a solid derivative of the nicotine methyl group for counting. Methyl iodide liberated by the action of hydriodic acid reacted with triethylamine to yield triethylmethylammonium iodide. Triethylamine was found to react quantitatively with methyl iodide, and is much less volatile than the trimethylamine used by earlier workers.[19]

The demethylation apparatus was similar to that described by Pregl.[18] Ground joints were introduced to facilitate disassembly of the apparatus for cleaning, and also to enable the reaction vessel to be used as a distilling flask for the concentration of the nicotine solution. A stopcock permitted the side arm to be closed off during the concentration. Nicotine hydrochloride equivalent to about 50 mg. of nicotine was placed in the reaction vessel, together with 45 mg. of ammonium iodide, two drops of 5% gold chloride solution, and 3 ml. of hydriodic acid (specific gravity 1.7). The gas-washing vessel contained 1.5 ml. of the cadmium sulfate–sodium thiosulfate solution described by Pregl, to remove iodine and hydrogen iodide. The receiver contained a 5% ethanolic solution of triethylamine and was cooled in a solid carbon dioxide–methyl cellosolve bath.

Because of its relative insolubility it was not found practical to use the nicotine picrate as such for demethylation. Instead, it was dissolved in dilute sodium hydroxide and the nicotine was recovered by azeotropic distillation as before. The acid distillate was then concentrated, with the concentration being completed in the reaction flask.

After the addition of the other reactants as described above, the flask was connected to the demethylation apparatus and heated[20] in the cupric oxide bath. A slow stream of nitrogen was passed in through the side arm, and the bath temperature was raised to 200° in 20 to 25 minutes. It was then raised rapidly to 350–360° and held there for 45 minutes. The heat was removed and the sweeping continued until the reaction flask had cooled. The receiving tube was then taken off, corked tightly, and allowed to stand overnight at room temperature. In the morning the alcohol and excess amine were removed by evaporation. A white, crystalline residue of triethylmethylammonium iodide was recovered in 55–70% of the theoretical yield based on nicotine.

Anal. Calcd. for $C_7H_{18}NI$: I, 52.20. Found: I, 52.31.

Administration of C^{14}-Methionine to *Nicotiana rustica*.—As nicotine synthesis has been shown to take place in the roots of tobacco,[21] it appears advantageous to allow the plant to absorb the methionine from aqueous solution through the roots. First, however, it was necessary to ascertain whether or not the methionine was absorbed by the plant, and whether it was destroyed by microörganisms in the medium before there was opportunity for its absorption.

To test the uptake of methionine 3 mg. of the amino acid was administered in the nutrient medium to each of six plants. After 24 hours the plants were removed from the solution and the roots were rinsed into the residual medium, which was then analyzed for methionine by the colorimetric method of McCarthy and Sullivan.[22] In the 24-hour period 67–80% of the methionine was absorbed. No evidence of bacterial growth was observed during this time, but as a check an experiment was carried out in which the medium was innoculated by the addition of a few root fragments. After 24 hours the analysis showed no detectable loss of methionine.

In preliminary experiments with radioactive methionine, 2 mg. of methionine, containing 10^5 counts per minute, was given to each plant for 24 hours. This administration period, however, resulted in very low activity (about 25 c.p.m. over background at infinite sample thickness) in the isolated nicotine picrate. To avoid the errors inherent in counting such low activities the time in contact with the isotope was extended to seven days. The nicotine picrate isolated after this interval was found to be considerably more active, having a maximum specific activity, calculated for zero sample thickness, of the order of 4,000 c.p.m. per millimole. One recrystallization was sufficient to bring the activity to a constant value. The seven-day administration period was used in all subsequent experiments.

Administration of C^{14}-Formate to *Nicotiana rustica*.—No micromethod could be found by which formate in the nutrient medium could be analyzed with the degree of accuracy realized in the methionine determinations. Nevertheless, it was found possible to obtain semi-quantitative results using the colorimetric procedure of Grant.[23] One milligram of sodium formate in 50 ml. of nutrient medium was administered to each of a group of plants, and over a 48-hour period a quantity of the order of 50% was taken up, the amount varying somewhat with plant size. No loss by bacterial action, in flasks inoculated with plant root fragments, could be observed. To check further the uptake of formate by the plants an aliquot of one of the nutrient solutions was dried and counted. In less than three days from the start of the run, only about 1% of the administered radioactivity could be accounted for in this way. Thus by this time, at the latest, virtually all the formate had been absorbed by the plant.

In administering radioactive formate it was desirable, for purposes of comparison, to maintain the experimental conditions as close as possible to those prevailing during the methionine experiments. However, it was not possible to maintain constant the previous treatment of the plants, because of seasonal variations in light, temperature and other factors. A sample of C^{14}-formate was diluted so that 0.91 mg. of the sodium salt, containing 10^5 c.p.m., was given to each plant; the molar quantity and the total counts per minute were thus the same as in the case of methionine. The plants were grown as described previously for seven days. They were then worked up as before, and nicotine was again isolated as the dipicrate, counted, and demethylated.

Determination of Radioactivity.—All counts were made on a Nuclear Instrument and Chemical Corp. end-window counter on the top shelf of the counter assembly. The over-all efficiency was 7%, determined on a National Bureau of Standards carbon-14 standard.

The active methionine and formate samples were counted as "infinitely thin" layers on aluminum dishes 2.83 cm. in

(14) H. Schmid and M. Serrano, *Experientia*, **4**, 311 (1948).

(15) C. R. Smith, *Ind. Eng. Chem.*, **34**, 251 (1942).

(16) T. A. Henry, "The Plant Alkaloids," The Blakiston Co., Philadelphia, Pa., 1949, p. 37.

(17) J. Herzig and H. Meyer, *Monatsh.*, **15**, 613 (1894).

(18) F. Pregl, "Quantitative Organic Microanalysis," 4th Eng. Edn., The Blakiston Co., Philadelphia, Pa., 1946, pp. 156–160.

(19) S. Simonds, M. Cohn, J. P. Chandler and V. du Vigneaud, *J. Biol. Chem.*, **149**, 519 (1943).

(20) Heating with hydriodic acid causes a gradual etching of the glass, eventually rendering it fragile. It is not advisable to use the vessel more than six or seven times.

(21) R. F. Dawson, *Am. J. Botany*, **29**, 66, 813 (1942).

(22) T. E. McCarthy and M. X. Sullivan, *J. Biol. Chem.*, **141**, 871 (1941).

(23) W. M. Grant, *Anal. Chem.*, **19**, 206 (1947).

diameter. The samples of nicotine dipicrate and triethylmethylammonium iodide, which were less active, were counted on similar dishes and corrected to "infinite thinness" (maximum specific activity) from a self-absorption curve.

Results and Discussion

In Table I are shown the specific activities of the nicotine dipicrate, and its degradation product triethylmethylammonium iodide, isolated following the administration of C^{14}-labeled methionine and formate.

TABLE I

LOCATION OF RADIOACTIVITY IN THE NICOTINE MOLECULE

Compound administered	Run	Number of plants	Maximum specific activity (counts per minute per millimole) Nicotine dipicrate	Quaternary iodide
I, C^{14}-Methyl-methionine	1	39	4.28×10^3	4.42×10^3
	2	40	4.24×10^3	3.92×10^3
	3	39	7.53×10^3	7.60×10^3
II, C^{14}-Formate	1	25	9.6×10^2	9.3×10^2
	2	29	4.4×10^2	4.1×10^2

From these figures it can be seen that there has been considerable incorporation of the methyl carbon of methionine into the nicotine molecule. The recovery of the nicotine activity as the methyl group of the quaternary iodide ranged from about 93 to 103%, indicating that, within experimental error, all the activity is localized in the methyl carbon of nicotine. The reason for the relatively high values in Experiment I, 3 is not apparent, but the explanation may lie in seasonal influences, as the plants were grown at different times of the year.

On the basis of recent experiments formate, or some one-carbon compound which has been designated as "formate," appears to have an important role in methylation reactions. du Vigneaud and his co-workers, in experiments with rats using C^{14}, have shown that formate can act as a precursor of the methyl carbon of choline.[24] In view of the findings of Sakami[25] and of Weissbach, Elwyn and Sprinson,[26] it is probable that the conversion takes place by way of serine, as shown schematically

$$HC^*OOH + H_2N{-}CH_2{-}COOH \longrightarrow$$

"formate" glycine

$$HOC^*H_2{-}\underset{\displaystyle NH_2}{\underset{|}{CH}}{-}COOH \longrightarrow$$

serine

$$(H_2N{-}CH_2{-}C^*H_2OH) \longrightarrow$$

ethanolamine

$$[(C^*H_3)_3\overset{+}{N}{-}CH_2{-}C^*H_2OH]\,OH^-$$

choline

It is probable that other intermediates, as yet unknown, also enter into this transformation. Choline can transfer its methyl carbon, through betaine, to methionine.[27] On the other hand, methionine can supply a methyl carbon to sarcosine,[28] and Mackenzie[28] has shown that the methyl group of sarcosine can be oxidized to formate by rat liver homogenates.

Under normal conditions in the plant labile methyl groups must be synthesized from simpler compounds, as an exogenous source is unavailable. Kirkwood and Marion[7] recently fed C^{14}-formate to sprouting barley and isolated choline containing C^{14} in the methyl groups. It thus appears that in the plant, as well as in the animal, formate can act as a labile methyl precursor, and by analogy to the reactions in the animal it is probable that the labile methyl is then transferred as an entity to the methyl acceptor.[8] However, the possibility remains that the labile methyl is oxidized to formate and subsequently reduced during the transfer of the methyl carbon from donor to acceptor. If this were the case the formate pool in the plant could presumably act as a source of methyls, and the carbon of formate administered to tobacco plants would not necessarily pass through the methionine methyl group in its conversion to the methyl carbon of nicotine. Hence, formate administered to tobacco should result in a specific activity for the isolated nicotine at least as high as that obtained following methionine administration, because the dilution in the labile methyl pool would be bypassed to a great extent.

In an experiment designed to compare the rates of nicotine methyl formation from methionine and formate it is necessary to assume that the rates of uptake are about the same, that the size of the pools in the plant remains constant, and that there is no variation in the ratio of nicotine formed during the period of the experiment to that previously stored in the plant. It can be seen from Table I that there is in reality a variation in one or more of these factors, for the specific activity of the nicotine in Experiment I, 3 is about 75% higher than that in Experiments 1 and 2. It follows that any difference in the activity of the nicotine isolated subsequent to the administration of formate would have to be of the order of several hundred per cent. before any inferences as to pathways could be drawn.

It is evident from the data presented in Table I that there has been some incorporation of the formate carbon into nicotine. The low count in this case introduces relatively larger errors in comparison to those of the methionine experiments, and it is not possible to compare the specific activities of the nicotine and the quaternary iodide with the same degree of accuracy. For this reason it is not possible to state with finality that all of the activity in the nicotine resides in the methyl group, but it is apparent that most of it, at least, can be accounted for in that position. A comparison of the values obtained following the feeding of formate with those obtained after methionine administration reveals that the former are considerably lower. The lowest specific activity obtained for the methyl carbon in the methionine experiments (I, 2) is over four times as high as the higher obtained after formate administration (II, 1), whereas at the other extreme the value I, 3 is nearly nineteen

(24) V. du Vigneaud, W. G. Verly and J. E. Wilson, THIS JOURNAL, **72**, 2819 (1950).

(25) W. Sakami, *J. Biol. Chem.*, **176**, 995 (1948).

(26) A. Weissbach, D. Elwyn and D. B. Sprinson, THIS JOURNAL, **72**, 3316 (1950).

(27) J. A. Muntz, *J. Biol. Chem.*, **182**, 489 (1950).

(28) C. G. Mackenzie, *ibid.*, **186**, 351 (1950).

times in excess of II, 2. It appears, therefore, that when C^{14}-formate is fed to *N. rustica* the specific activity of the nicotine methyl carbon isolated is about one order of magnitude lower than that which results after feeding C^{14}-methylmethionine, under conditions maintained as nearly as possible constant.

The data presented above, in the opinion of the authors, constitute strong evidence for transmethylation in higher plants. The lower specific activities obtained using formate serve as an indication that the methionine methyl group is not oxidized to formate and then reduced during the transfer of the methyl carbon to nicotine. It may be inferred that the function of formate in transmethylation is, alternatively, that of a precursor in the biosynthesis of labile methyl groups, with serine acting as a possible intermediate. The final proof that the methionine methyl group is transferred as an entity to nicotine must await the completion of experiments involving double-labeling with carbon-14 and deuterium. These experiments are now being conducted in this Laboratory, and the results will be forthcoming at a later date.

In view of our results indicating transmethylation from methionine to nicotine, and the failure of Kirkwood and Marion[7] to demonstrate any methyl transfer from choline to hordenine in barley, it appears that further comparative studies would be profitable. The possible function of choline as a methyl donor in nicotine biosynthesis is now under investigation.

Acknowledgments.—The authors wish to express their thanks to the Eastman Kodak Co., of Rochester, New York, for financial support in the form of a fellowship to one of them (S. A. B.) They are also grateful to Dr. E. H. Lucas of the Department of Horticulture, Michigan State College, for his assistance in cultivating the plants; and to Dr. N. A. MacRae of the Canadian Department of Agriculture, Ottawa, for his generosity in furnishing the seeds.

EAST LANSING, MICHIGAN RECEIVED SEPTEMBER 13, 1951

[CONTRIBUTION NO. 535 FROM THE DEPARTMENT OF CHEMISTRY, INDIANA UNIVERSITY]

Reactions of Silane. I. The Alkylation and Alkoxylation of Silane

BY JOHN S. PEAKE, W. H. NEBERGALL AND YUN TI CHEN[1]

The apparatus and procedure for the preparation of silane by the lithium aluminum hydride reduction of tetrachlorosilane and the subsequent alkylation and alkoxylation of silane as a continuous process have been described. Tetraphenylsilane, tetraethylsilane, triethylsilane, diethylsilane, triisopropylsilane, tetra-2-naphthylsilane and tri-1-naphthylsilane were formed by the interaction of silane and the appropriate organolithium compound. Phenylsodium reacted with silane to form tetraphenylsilane and sodium hydride. Attempts to bring about reactions between silane and Grignard reagents, phenylcalcium iodide, diphenylcalcium and diethylzinc were unsuccessful. Silane has been found to react with various alcohols in the presence of alkoxide ions to produce tetraalkoxysilanes and hydrogen. Tetraethoxysilane, tetra-*n*-propoxysilane and tetra-*n*-butoxysilane were prepared by the reaction of silane with the appropriate alcohol; however, no reaction was observed when silane was passed through methanol containing lithium methoxide.

No method for the direct alkylation or alkoxylation of monosilane, SiH_4, has previously appeared in the literature. However, organolithium compounds have been used to replace hydrogen attached to silicon in such alkylsilanes as R_3SiH,[2] R_2SiH_2[3] and $RSiH_3$,[4] which reactions result in the formation of tetraalkylsilanes and lithium hydride.

As a result of the present investigation one may write a general equation to represent the replacement of hydrogen attached to silicon by the use of organolithium compounds.

$$R_ySiH_{4-y} + (4-y)\,R'Li \longrightarrow R_ySiR'_{4-y} + (4-y)\,LiH$$

In the above equation R may be an alkyl or aryl radical and y may have values from zero to three.

The importance of the solvent in these substitution reactions has been noted previously[2,4] and is further verified in this report. When ethyl ether is employed as the reaction medium for the interaction of RLi compounds and organosilicon hydrides, tetrasubstitution is favored, but when low boiling petroleum ether is the solvent, trisubstitution appears to be the general rule. For example, ethyllithium reacts with silane to form tetraethylsilane in ethyl ether, whereas triethylsilane is the most highly substituted product formed when the reaction medium is petroleum ether.

It was observed that isopropyllithium in excess reacted with silane in petroleum ether to produce triisopropylsilane almost exclusively. Restriction to trisubstitution here does not necessarily result from the nature of the solvent in view of the fact that others have reported[5] possible steric effects of the isopropyl group in attempting to synthesize tetraisopropylsilane from tetrachlorosilane. Another case of steric hindrance was encountered in the present work in that tri-1-naphthylsilane was found to be the sole product of the reaction of an excess of 1-naphthyllithium with silane in ethyl ether. It is interesting to note, however, that tetrasubstitution was readily effected by the action of 2-naphthyllithium upon silane.

The possibility of employing other organometallic compounds in addition to those of lithium was in-

(1) Part I from a thesis to be submitted by Yun Ti Chen to the Graduate School of Indiana University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

(2) R. N. Meals, THIS JOURNAL, **68**, 1880 (1946); H. Gilman and S. P. Massie, *ibid.*, **68**, 1128 (1946); H. Gilman and H. W. Melvin, Jr., *ibid.*, **71**, 4050 (1949); R. A. Benkeser and F. J. Riel, *ibid.*, **73**, 3472 (1951).

(3) Unpublished work carried out in this Laboratory.

(4) W. H. Nebergall, THIS JOURNAL, **72**, 4702 (1950).

(5) H. Gilman and R. N. Clark, *ibid.*, **69**, 1499 (1947).