[CONTRIBUTION FROM THE CHEMISTRY DIVISION, ARGONNE NATIONAL LABORATORY]

The Synthesis of Tritium-labeled Methyl Iodide and Acriflavine[1]

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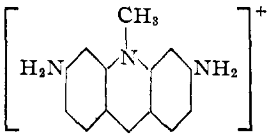
The preparation of 10-methyl-*t*-3,6-diaminoacridinium chloride (acriflavine) from a hydrogen-tritium mixture with a radiochemical yield of better than 60% is described. The synthesis is based on a preparation of methyl-*t* iodide from tritium gas with a radiochemical yield of 78% by reduction of methyl formate with tritiated lithium borohydride, and treatment of the reaction product with hydriodic acid. Acriflavine, free of non-methylated material, is obtained, without resort to customary purification techniques, from the reaction of 3,6-diacetylaminoacridine and methyl iodide in a sealed tube at 110°, by hydrolysis and filtration.

For an investigation[3] of the effect of irradiation of cell nuclei on cell reproduction, a supply of acriflavine (10-methyl-3,6-diaminoacridinium chloride (I) or its hydrochloride) containing about one curie of tritium per gram was desired. To minimize the possible loss of tritium by exchange *in vivo*, attention was confined to the introduction of tritium in the methyl group *via* alkylation of 3,6-diacetylaminoacridine with a tritiated methylating agent and hydrolysis with hydrochloric acid. Conventional procedures for the methylation, involving use of dimethyl sulfate[4] or methyl *p*-toluenesulfonate[5,6] in nitrobenzene solution, were unsatisfactory because the reactions are incomplete[7] and purification[6,8,9] of acriflavine from the non-methylated material (proflavine) is difficult. A brief investigation showed however, that acriflavine containing 1% or less of proflavine could be obtained, simply by hydrolysis and filtration, when the methylation was performed in the absence of solvent with a slight excess of methyl iodide in a sealed tube at 110°.



I

Published procedures for the preparation of tritiated methyl iodide, or its precursor, methanol, also were not suitable for the present synthesis. In the catalytic reduction[10] of methyl formate efficient utilization of tritium is difficult since a tenfold excess of the hydrogen-tritium mixture is used; in the reaction[11] of tritiated water with diazomethane isotopic dilution is excessive. It seemed that these difficulties could be avoided if tritiated lithium borohydride were prepared[12] and used to reduce methyl formate, as shown in equations 1 and 2. Since the distribution of tritium at equilibrium

$$\mathrm{LiBH_4 + H_2\text{-}}t \overset{200°}{\longleftrightarrow} \mathrm{LiBH_4\text{-}}t + \mathrm{H_2} \qquad (1)$$

$$\mathrm{LiBH_4\text{-}}t + \mathrm{2HCOOCH_3} \longrightarrow \mathrm{LiB(OCH_3\text{-}}t)_4 \qquad (2)$$

in the exchange reaction is approximately statistical, more than 90% of the tritium in hydrogen gas can be incorporated into lithium borohydride with only tenfold isotopic dilution. The discovery that methyl iodide could be prepared in excellent yield, without isolation of methanol, simply by removing solvent from the reduction product and heating the residue with hydriodic acid, made the adoption of this procedure particularly attractive.

The results of three syntheses employing these reactions at successively higher concentrations of tritium are summarized in Table I. In the first run, the methyl iodide was used in exploratory experiments; if the procedure employed in the other runs had been followed, the radiochemical yield of acriflavine would have been about 68%. The lower yields obtained in the second, and particularly in the third, runs are attributed not to a direct effect of radiation or difference in zero-point energy on the course of the reaction, but rather to the radiation-induced formation of some contaminant that, by reaction with the borohydride, adversely affected its surface and reducing properties. This hypothesis is supported by the fact that there was a significant increase in the volume of gas during the exchange reaction at the highest concentration of tritium. The contaminant, which might well be water, is presumably formed, under the influence of radiation, either from the glass, or from a reaction of the glass with hydrogen. Whether the contamination occurred during, or prior to, the exchange cannot be determined from the present experiments, however, since the gas used for the exchange was freshly prepared only in the first run and had been stored in glass for successively longer periods in the second and third runs. Although it cannot, therefore, be determined whether the yields in the latter runs are characteristic of the method at high concentrations of tritium, it can be stated that the yields in the first run, 78% for methyl iodide and about 68% for acriflavine, are representative of the method if freshly prepared tritium gas is used at tracer concentrations. In the light of these results, the advisability of using freshly prepared or purified samples in all reactions with tritium gas cannot be emphasized too strongly.

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[2] Department of Anatomy, University of Chicago. Appointment aided by grants from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council and from the Alexander and Margaret Stewart Cancer Research Fund.

[3] This effect will be investigated by Prof. P. P. H. De Bruyn, Department of Anatomy, University of Chicago.

[4] E. Grandmougin and K. Smirous, *Ber.*, **46**, 3431 (1913).

[5] L. Benda, *ibid.*, **45**, 1796 (1912).

[6] A. Albert and B. Ritchie, *J. Chem. Soc.*, 458 (1943).

[7] Dr. F. W. Morthland, unpublished work, has shown that the extent of methylation with methyl *p*-toluenesulfonate is further decreased by the presence of impurities difficult to remove in preparations on a radiochemical scale.

[8] J. Marshall, *Quart. J. Pharm. and Pharmacol.*, **1**, 514 (1934).

[9] P. Gaillot, *Bull. soc. chim.*, [V] **1**, 796 (1934).

[10] D. Harmon, T. D. Stewart and S. Ruben, THIS JOURNAL, **64**, 2293 (1942).

[11] L. Melander, *Arkiv Kemi*, **3**, 525 (1951).

[12] W. G. Brown, L. Kaplan and K. E. Wilzbach, THIS JOURNAL, **74**, 1343 (1952).

TABLE I

SUMMARY OF SYNTHESES OF TRITIUM-LABELED METHYL IODIDE AND ACRIFLAVINE

Compound	Run[a] I	II	III
Hydrogen–tritium mixture			
Quantity, mmole			
Initial	0.56	0.82	0.50
Final	0.56	0.83	0.65
Activity, curies			
Initial	0.00576	0.644	20.88
Final	0.00053	0.071	5.34
Lithium borohydride			
Quantity, mmoles	6.38	5.18	3.72
Activity,[b] curies	0.00523	0.573	15.54
Radiochemical yield, %	90.8	89.0	74.3
Methyl iodide			
Quantity, mmoles	23.7	19.5	15.1
Activity, curies	0.00450	[c]	[c]
Radiochemical yield, %	78.1	[c]	[c]
Acriflavine			
Quantity, mmoles	......	17.2	14.0
Activity, curies	......	0.395	7.82
Radiochemical yield, %	......	61.4	37.5
Molecules per atom T	......	1255	51.7

[a] Run: hours of exchange at 200°, mmoles methyl formate reduced, mmoles diacetylaminoacridine used. I: 67, 13.44, ---; II: 90, 10.46, 19.2; III: 137, 16.23, 15.0. [b] Obtained by difference. [c] Not determined.

Procedure

Since the three runs were conducted in much the same way, only one, the second, will be described in detail; the quantities and conditions used in the other runs can be found in Table I.

Tritium Assay.—Tritium was determined[13] in gaseous samples by measurement of the ion current; the zinc fusion technique[14] was used for the preparation of gas samples from organic compounds.

Exchange Reaction.—The vessel used for the exchange reaction, the reduction of methyl formate, and the preparation of methyl iodide consisted of a 50-ml. bulb sealed to a micro-condenser having at its upper end a female ground glass joint and a side arm with a stopcock and a joint. An ether solution of purified[15] lithium borohydride was filtered into the reaction flask through the sintered glass disk of a long stem funnel. After complete removal of solvent, by evacuation at 200°, the weight of hydride was found to be 0.113 g. The reaction flask was then mounted in a horizontal position on a Toepler pump and 18.28 cc. of hydrogen containing 644 millicuries of tritium was introduced. The exchange reaction was effected by immersing the bulb of the flask in a bath maintained at 200°. The extent of the exchange was determined, at suitable intervals, by cooling the vessel to room temperature, expanding the gas into a small calibrated volume, and expanding this aliquot into an ionization chamber for determination of tritium. After 97 hours, the tritium content of the gas was 71 millicuries. The amount of tritium in the borohydride, obtained by difference, was 573 millicuries, 89% of that initially present in the gas.

Reduction.—The tritiated lithium borohydride was dissolved in about 10 ml. of tetrahydrofuran, distilled into the flask from lithium aluminum hydride. The solution was frozen and 0.629 g., a 1% excess, of methyl formate was introduced; a larger excess was avoided since there is a distribution of methoxyl groups between the reduction product and unreacted ester. The reduction was effected by gradually warming the mixture, maintaining it at 0° for 30 minutes and at 20° for one hour. The solvent was then transferred, by distillation *in vacuo*, to a similar reaction vessel containing 20 mg. of non-labeled borohydride to reduce unreacted methyl formate. The solvent was completely removed from both reduction products by evacuation at 60°.

Preparation of Methyl Iodide.—Each reaction product, above, was treated with 25 ml. of hydriodic acid (Merck and Co., for methoxyl determination), added through a dropping funnel mounted on the condenser and having a stem long enough to project into the bulb of the reaction flask. The condenser was cooled with a flow of water, and the solution was heated at 90° for two hours while helium, at a rate of about 30 cc./min., was passed through the funnel, the solution, a trap cooled in liquid nitrogen, and a pressure outlet. The trap was then warmed to −95°, with a melt formed by the addition of liquid nitrogen to toluene, and evacuated to remove material more volatile than methyl iodide. Less volatile material remained behind when the trap was warmed to −63°, with chloroform–liquid nitrogen, and the methyl iodide was allowed to distil into a storage vessel. The quantities obtained from the reductions with labeled and non-labeled lithium borohydride were 2.775 and 0.106 g., respectively. The total yield of methyl iodide, 20.3 mmoles, was 97%, based on methyl formate. The products were largely solid at −65°; the melting point of methyl iodide is −64.4°. The products in this run, and in run III, were not analyzed because of the difficulties involved in obtaining a representative sample of a volatile, highly radioactive liquid; the methyl iodide in run I was found to contain 8.60% carbon and 2.12% hydrogen (theoretical values, 8.46%, 2.13%) and 78% of the tritium initially present in the gas.

Preparation of 3,6-Diacetylaminoacridine.—The base precipitated by the gradual addition of 750 ml. of ammonium hydroxide to a stirred solution of 50 g. of 3,6-diaminoacridine sulfate (Eastman Kodak Co.) in 10 liters of water was refluxed with 65 cc. of acetic anhydride and 6.5 g. of anhydrous sodium acetate for 30 minutes. While the mixture was still hot, water was added until the solid just dissolved. The acetate of 3,6-diacetylaminoacridine, which separated on cooling, was dissolved in 5 liters of water and converted to the free base by the gradual addition of 500 ml. of ammonium hydroxide to the stirred solution. The 3,6-diacetylaminoacridine obtained had a melting point of 270°, and gave a negative test for free amino groups. Before use, the product was ground in a mortar and dried *in vacuo* at 100°.

Methylation.—An H-shaped reaction vessel was used for methylations: one arm, of 15 mm. o.d. tubing, was sealed to a male ground glass joint; the other arm, of 6 mm. o.d. tubing, was made from a ground glass joint with stem sealed at both ends. The tube was constructed to have a volume (36 cc.) such that the pressure of methyl iodide would be 3–4 atmospheres when methylation was complete. A sample of 3,6-diacetylaminoacridine, 4.526 g., 15.43 mmoles, was weighed into the larger arm of the reaction tube, and a fraction of methyl-*t* iodide described above, 2.775 g., 19.53 mmoles, was added to the other arm by distillation *in vacuo*. The reaction vessel was then sealed off and heated at 50° for 16 hours. At the end of this period the extent of methylation was determined, by the decrease in the column of liquid methyl iodide, to be about 90%; the methylation was completed by heating the tube at 110° for 48 hours. After the smaller arm had been cooled in liquid nitrogen to condense methyl iodide, the stem of the ground glass joint was snapped off, and the tube was evacuated at 100° to ensure complete removal of volatile material. The weight of the solid product was 6.682 g.; the increase in weight was equivalent to 98.4% methylation. The volatile material, purified by distillation, yielded 0.616 g. of methyl iodide. This methyl iodide, 4.35 mmoles, was heated with 1.116 g., 3.81 mmoles, of 3,6-diacetylaminoacridine in a similar reaction vessel with a volume of 9 cc. The product of the second methylation weighed 1.641 g., corresponding to 97.2% methylation.

Hydrolysis.—The total product of the two methylations, 8.323 g., was transferred to a flask with two necks, one of which contained a sintered glass disk. In an atmosphere of nitrogen, the solid was dissolved in 450 ml. of hot, dilute (1:10) hydrochloric acid, and the solution was filtered. Acriflavine hydrochloride was then precipitated by addition

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[13] K. E. Wilzbach, A. R. Van Dyken and L. Kaplan, *Anal. Chem.*, **26**, 880 (1954).

[14] K. E. Wilzbach, L. Kaplan and W. G. Brown, *Science*, **118**, 522 (1953).

[15] W. D. Davis, L. S. Mason and G. Stegeman, THIS JOURNAL, **71**, 2777 (1949).

of 550 ml. of concentrated hydrochloric acid and refrigeration of the solution. The weight of product, collected by filtration and dried *in vacuo*, was 5.088 g., 89.0% of that expected from the total quantity of diacetylaminoacridine used. The amount of tritium in the product was found to be 395 millicuries, 61.4% of that initially in the hydrogen gas. The chloride content, determined gravimetrically,[16] was 23.77%; the theoretical value for acriflavine hydrochloride is 23.94%. The picrate melted at 245–246°; the melting point reported[17] for the picrate of acriflavine is 244°. Analysis of product by filter paper partition chromatography, using the method of Lederer[18] modified by developing the chromatograph with *n*-octyl alcohol saturated with 1.5 *N* ammonium hydroxide, and calibrating with known mixtures, indicated the presence of 1% of proflavine.

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[16] R. K. Snyder, *Bull. Natl. Formulary Comm.*, **9**, 248 (1941).

[17] A. Bolliger, *Quart. J. Pharm. and Pharmacol.*, **13**, 1 (1940).

[18] M. Lederer, *Anal. Chim. Acta*, **6**, 267 (1952).

LEMONT, ILLINOIS

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF COLUMBIA UNIVERSITY]

Studies on α-Pyridones. II. Derivatives of Pyrindine

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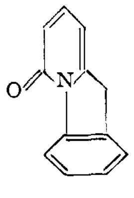
The action of mineral acids on 2-(2′-oxocyclohexyl)-methyl-6-chloronicotinic acid (I) leads to 6-(4′-carboxy)-butyl-2-hydroxy-5-oxo-6,7-dihydro-1,5H-pyrindine (II). This rearrangement, which provides a novel route to derivatives of pyrindine, is interpreted as involving the acid-catalyzed cyclization of the keto acid I to a spiro-β-diketone (C) followed by cleavage of C to the keto acid II. The structure of II follows from chemical and spectral data and is proven by an unequivocal synthesis starting from ethyl 2-bromomethyl-6-chloronicotinate. Ultraviolet absorption data of a number of α-pyridone derivatives in the pyridine and pyrindine series are provided.

Our exploration[2] of possible routes to α-pyridone derivatives of benzo[b]pyrrocoline (A) has led us to the examination of the action of mineral acids on 2-(2′-oxocyclohexyl)-methyl-6-chloronicotinic acid (I). The present paper furnishes chemical and spectroscopic evidence for the formulation I → II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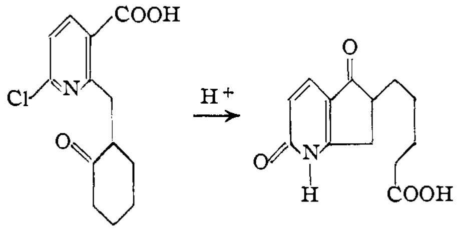
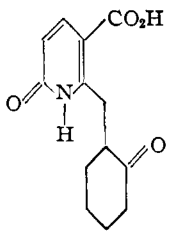


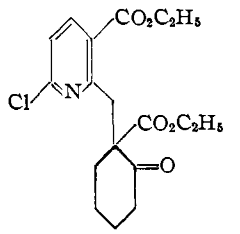
I II

as the over-all result of the action of mineral acids on I. An interpretation of the course of this rearrangement, which provides a route to derivatives of 1,5H-pyrindine and bears on the more general question of acid catalysis in the condensation of ketones with carboxylic acids, is also presented. Some correlations between structure and ultraviolet absorption spectra among α-pyridones are discussed.

2-(2′-Oxocyclohexyl)-methyl-6-chloronicotinic acid (I) was obtained from the previously synthesized[2] ethyl 2-(1′-carbethoxy-2′-oxocyclohexyl)-methyl-6-chloronicotinate (III) by alkaline hydrolysis and decarboxylation. Ultraviolet and infrared (see Experimental) absorption spectra leave no doubt concerning the structure of this α-chloronicotinic acid derivative. I formed a 2,4-dinitrophenylhydrazone whose ultraviolet spectrum (λ_{max} 368 mμ) is fully consistent with the structure shown. The action of either 85% phosphoric acid or of a mixture of hydrochloric acid and acetic acid on I gave a high melting (m.p. 263–264°) crystalline substance of formula $C_{13}H_{15}NO_4$. It soon became apparent that a simple α-pyridone[3] formulation (B) for this ketoacid was untenable. For example, the substance formed a deep red 2,4-dinitrophenylhydrazone (λ_{max} 397 mμ) indicative of a high degree of conjugation for the hydrazone group. In view of this and of other data described below, the possibility of a deep-seated rearrangement during the acid treatment was considered and the structural hypothesis II was advanced for the substance, m.p. 263–264°.



B



III

The presence of an α-pyridone structure in II was evident from its infrared spectrum showing the characteristically strong band at 6.05 μ; at this point the ultraviolet spectrum of II (Fig. 1) was of little diagnostic value due to the limited spectral-structural correlations in the α-pyridone series now in existence.[2] Esterification of the keto acid II using alcoholic hydrochloric acid gave the corresponding ethyl and methyl esters (IV and V, respectively), of ultraviolet spectra (Fig. 1) similar to that of the parent carboxylic acid. Alkylation of the sodio salt of the α-pyridone V with methyl iodide gave the N-methyl-α-pyridone VI; VI is obtainable also from the carboxylic acid II upon treatment with diazomethane in ether–methanol. This example of N-alkylation of an α-pyridone with diazomethane is of interest, inasmuch as it has been stated[4] that

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[1] David W. and Ellen A. Ferguson Fellow, 1953–1954. From part of the Ph.D. Thesis of A. P. Paul.

[2] F. Ramirez and A. P. Paul, *J. Org. Chem.*, **19**, 183 (1954).

[3] The conversion of α-chloropyridines into α-pyridones by mineral acids was recently described (ref. 2).

[4] H. S. Mosher in "Heterocyclic Compounds," edited by R. C. Elderfield, John Wiley & Sons, Inc., New York, N. Y., Vol. I, p. 435, 534 (1950).