say, the volume of diluent placed in the mixing reservoir should at least equal the volume of eluate to be collected. Furthermore, if the total volume of solution leaving the reservoir exceeds twice the original volume in the reservoir, the gradient effect of any further elution is negligible.

A more limited form of expression for gradient elution was derived empirically by Donaldson, *et al.*,[3] from experimental data.

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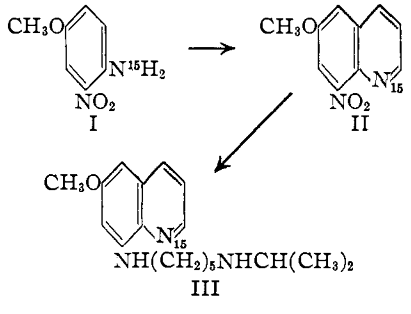
Synthesis of Pentaquine Labeled in the Quinoline Ring with N^{15} [1]

BY ROBERT C. ELDERFIELD,[2] LELAND L. SMITH AND ELEANOR WERBLE

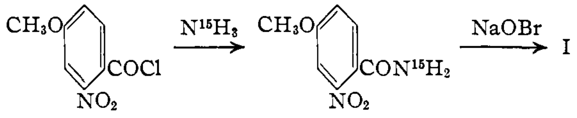
RECEIVED NOVEMBER 4, 1952

In the preceding paper the preparation of pentaquine [6-methoxy-8-(5-isopropylaminopentylamino)-quinoline] carrying N^{15} in each of the two side chain positions was described.[3] The results of a study of the excretion products of these two labeled drugs when fed to monkeys are described in an accompanying article.[4] In view of the inconclusive nature of the latter studies insofar as the physiological disposition of the drug is concerned, it was felt that a similar study of pentaquine labeled with N^{15} at the quinoline nitrogen was mandatory and might be productive of more useful information. Accordingly we wish to report at this time the synthesis of this substance. The physiological studies with the drug are under way and will be reported in a later communication.

The obvious route to the synthesis of the desired drug involves preparation of 4-methoxy-2-nitroaniline (I) carrying N^{15} in the amino group. By conventional methods 6-methoxy-8-nitroquinoline (II)[5,6] and pentaquine (III)[7] labeled at the ring nitrogen are then easily available.



A logical means for the introduction of N^{15} into I appeared to be at hand in the reaction of 4-methoxy-2-nitrochlorobenzene (IV) with potassium phthalimide enriched with N^{15}. In preliminary experiments *o*-nitrochlorobenzene reacted in good yield with potassium phthalimide in boiling dimethylformamide to yield *o*-nitroaniline after hydrolysis. However, when the same reaction was attempted with IV, the deactivating effect of the methoxyl group was sufficiently great that the analogous reaction was completely prevented. Use of higher boiling solvents or substitution of bromine or iodine for the chlorine in IV were without effect. Therefore another route to I was employed as shown by the formulas



Pentaquine monophosphate was obtained in overall yield of 25% from V by this procedure.

p-Toluidine was nitrated according to Nolting and Collin[8] to yield 4-amino-2-nitrotoluene in 65% yield. This was diazotized to 4-hydroxy-2-nitrotoluene (VI)[9] in 36% yield. Methylation of VI with dimethyl sulfate[9] gave 4-methoxy-2-nitrotoluene in 88% yield. Permanganate oxidation of the latter according to Ullmann and Dootson[10] gave 4-methoxy-2-nitrobenzoic acid. Action of N^{15}-ammonia on the acid chloride of 4-methoxy-2-nitrobenzoic acid gave the amide, m.p. 160–162° from aqueous alcohol. (*Anal.* Calcd. for $C_8H_8N_2O_4$: C, 49.0; H, 4.1; N (normal N), 14.3. Found: C, 48.19; H, 4.4; N, 14.3, 14.6). By degradation of the amide with sodium hypobromite I was obtained in 66% yield.

The pentaquine monophosphate was enriched by 19.6 atoms % excess N^{15}.[11]

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(1) The work here reported was done under a grant from the National Institutes of Health to Columbia University.

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(3) A. H. Blatt and Norma Gross, THIS JOURNAL, **75**, 1245 (1953).

(4) R. C. Elderfield and L. L. Smith, *ibid.*, **75**, 1022 (1953).

(5) I. T. Strukov, *Org. Chem. Ind.* (*U.S.S.R.*), **4**, 523 (1937).

(6) H. S. Mosher, W. H. Yanko and F. C. Whitmore, *Org. Syntheses*, **27**, 48 (1947).

(7) N. L. Drake, *et al.*, THIS JOURNAL, **68**, 1529 (1946).

(8) E. Nolting and A. Collin, *Ber.*, **17**, 261 (1884).

(9) D. G. Harvey and W. Robson, *J. Chem. Soc.*,, 97 (1938).

(10 F. Ullmann and P. Dootson, *Ber.*, **51**, 9 (1918).

(11) The isotopic nitrogen analysis was done by Dr. D. Rittenberg of the College of Physicians and Surgeons of Columbia University to whom we wish to express our appreciation.

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Synthesis of Pentaquine Labeled in the Side Chain with N^{15} [1]

BY A. H. BLATT AND NORMA GROSS

RECEIVED NOVEMBER 4, 1952

In order to permit the study of the physiological disposition of pentaquine [6-methoxy-8-(5-isopropylaminopentylamino)-quinoline (I)] described by Elderfield and Smith[2] we prepared samples of pentaquine in which (a) the terminal nitrogen atom of the side chain and (b) the nitrogen atom attached to the 8 position of the quinoline ring was labeled with N^{15}. (For convenience these substances are designated pentaquine-N^{15}(T) and pentaquine-N^{15}(8), respectively.) The synthesis of the third isomer, in which the ring nitrogen atom is

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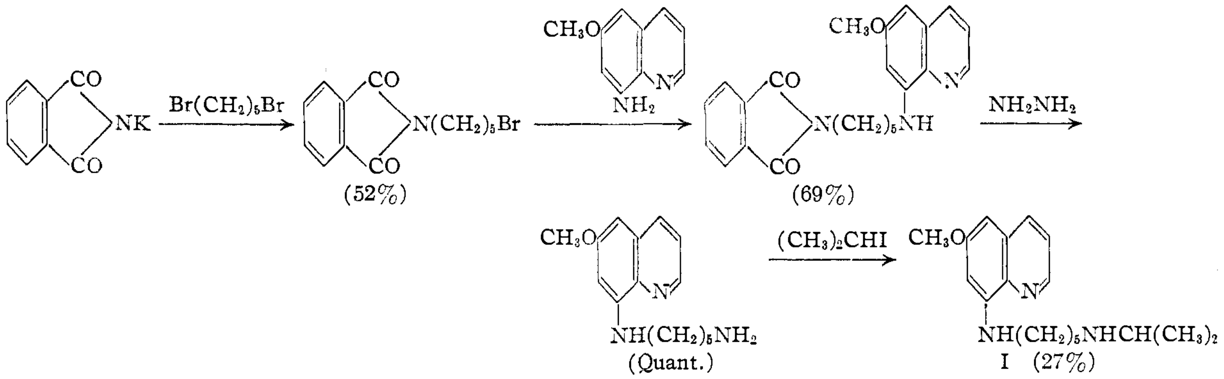
(1) The work reported in this note was done under a grant from the National Institutes of Health to Queens College.

(2) R. C. Elderfield and L. L. Smith, THIS JOURNAL, **75**, 1022 (1953).

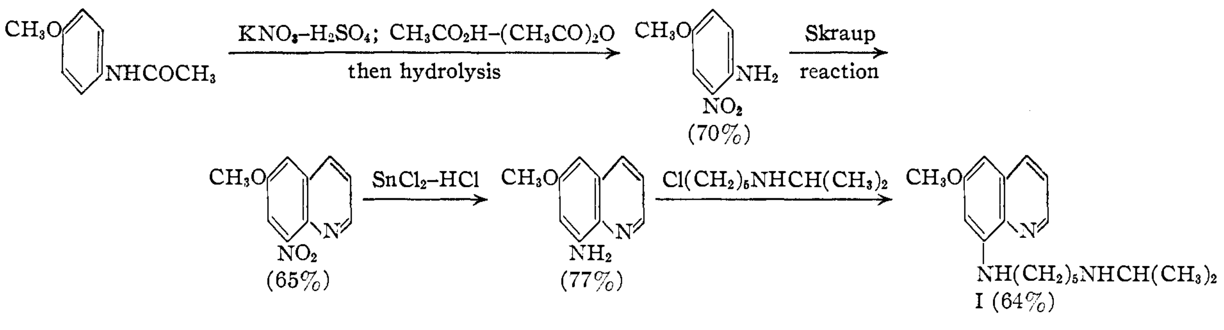
labeled with N^{15}, is described in the succeeding note by Elderfield, Smith and Werble.[3]

The synthesis of pentaquine-N^{15}(T) involved the following reactions starting with potassium phthalimide containing 62 atom per cent. excess N^{15}.



Our over-all yield in the first three reactions was 36%, compared with 39% obtained by Drake[4] whose directions were used. The final reaction, the attachment of the isopropyl group, had not been done before. Reductive alkylation with acetone gave erratic results so we alkylated with isopropyl iodide, following a procedure developed by Baldwin[5] for the corresponding alkylation with *n*-butyl iodide. The pentaquine-N^{15}(T) obtained as the monophosphate contained 20.5 atom per cent. excess N^{15} and had a 98% homogeneity by counter-current extraction.

The synthesis of pentaquine-N^{15}(8) involved the following series of reactions, in which the N^{15} was introduced in the first reaction by the use of potassium nitrate containing 62 atom per cent. excess N^{15}.



The over-all yield was 22.5%, as compared with an over-all yield of 24.7% for the same reactions as reported in the literature. The pentaquine-N^{15}(8) contained 20.0 atom per cent. N^{15}.

With one exception, available procedures[6] could be used for all the reactions in the synthesis of pentaquine-N^{15}(8). The exception is the first reaction, the nitration of acetyl-*p*-anisidine. The most satisfactory procedure for this nitration[6a] uses an excess (1.6 equivalents) of 70% nitric acid since this is less expensive than acetyl-*p*-anisidine. Concentrated nitric acid enriched in N^{15} was not available to us and we chose to use the easily handled N^{-15}-enriched potassium nitrate rather than to concentrate the dilute nitric acid. We also worked out, with unlabeled materials, conditions for the most efficient use of the nitrating agent since it is vastly much more expensive than the acetyl-*p*-anisidine. Our results, because of their possible general interest, are given in brief form.

1. Potassium nitrate and acetic acid will not nitrate acetyl-*p*-anisidine. A mineral acid is essential. Sulfuric acid is the obvious choice and it was used in all subsequent experiments.
2. Sulfuric acid cannot be used as the solvent for the nitration, for in this solvent the nitro group takes the position *ortho* to the methoxyl group. Acetic acid is obviously the solvent to choose and it was used in all subsequent experiments.
3. The nitrating agent (potassium nitrate and sulfuric acid) must be added to the solution of acetyl-*p*-anisidine in acetic acid. Addition of acetyl-*p*-anisidine in acetic acid to the nitrating agent results in the introduction of the nitro group in the *ortho* position to the methoxyl group.
4. A mixture of potassium nitrate, sulfuric acid and sufficient water to approximate 70% nitric acid is a satisfactory nitrating agent. However, some of the potassium nitrate does not dissolve and the loss would be prohibitively wasteful and expensive with potassium nitrate containing N^{15}.
5. A mixture of potassium nitrate and sulfuric acid to which sufficient water has been added to dissolve the salt is satisfactory for nitration if the acetyl-*p*-anisidine is dissolved in acetic acid plus

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[3] R. C. Elderfield, L. L. Smith and E. Werble, *ibid.*, **75**, 1245 (1953).

[4] Private communication from Dr. N. L. Drake.

[5] A. W. Baldwin, *J. Chem. Soc.*, 2959 (1929).

[6] (a) Nitration; directions of H. S. Mosher, W. H. Yanko and F. C. Whitmore, made available to us by Dr. R. C. Elderfield. (b) Skraup reaction; H. S. Mosher, W. H. Yanko and F. C. Whitmore, *Org. Syntheses*, **27**, 48 (1947). (c) Reduction; directions of T. A. Williamson, made available to us by Dr. R. C. Elderfield. (d) Attachment of side chain; N. L. Drake, *et al.*, THIS JOURNAL, **68**, 1529 (1946).

enough acetic anhydride to convert the added water to acetic acid.

6. It is more economical to use a slight excess of nitrating agent than to take the equivalent amount and then separate unreacted acetyl-*p*-anisidine from the nitration product.

With the information from these experiments we set up a procedure for nitrating acetyl-*p*-anisidine in acetic acid–acetic anhydride solution with a solution of potassium nitrate, sulfuric acid and water. The procedure gave satisfactory results as a comparison with the results of Mosher, Yanko and Whitmore[6a] shows.

	Equivalents of NO_3^-	Yield, % Based on acetyl-*p*-anisidine	Based on NO_3^-
Mosher, Yanko, Whitmore	1.6	85	57
This article	1.09	77	70

A more complete description of our work with full experimental details is available in microfilm.

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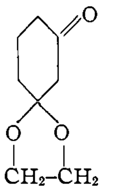
Reactions of the Monoethylene Ketal of 1,3-Cyclohexanedione

By Marshall W. Cronyn and Gregor H. Riesser

Received October 31, 1952

The reactions of the monoethylene ketal of 1,3-cyclohexanedione[1] (I) have been investigated in more detail in an effort to obtain certain 3-substituted cyclohexanones which could be of value as intermediates in the preparation of 5-substituted morphans.



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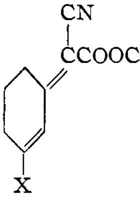

II, X = NH_2
III, X = $NHCH_2C_6H_5$
IV, X = OH
V, X = OCH_2CH_2OH

Reaction of the monoethylene ketal (I) with ethyl cyanoacetate in alcoholic ammonia did not give the expected Guareschi imide,[2] as shown both by its insolubility in dilute alkali and by its elemental analysis, $C_{11}H_{14}N_2O_2$. The analysis and the ultraviolet absorption spectrum indicated that the product, obtained in 82–90% yield, was ethyl α-cyano-3-amino-2-cyclohexene-$\Delta^{1,\alpha}$-acetate (II). Evidence for the structure of II was obtained by hydrolysis to the corresponding enol (IV), whose absorption spectrum was quite similar, and by the independent synthesis of both II and IV.

The enol (V) was synthesized by condensation of the monoethylene ketal (I) with ethyl cyanoacetate in chloroform with acetic acid and ammonium acetate.[3] The crude material obtained by distillation of the Knoevenagel reaction product was a mixture of the enol (V) and the corresponding cyclic ketal.

When the enol ether (V) was treated with alcoholic ammonia there was an immediate reaction and the eneamine (II) was obtained. Thus, under the Guareschi conditions, II must be obtained from the monoethylene ketal (I) by means of a Knoevenagel type reaction followed by opening of the cyclic ketal to the enol ether (V) and 1,6-addition of ammonia with elimination of the ethylene glycol.

As would be expected of a vinylog of an amide, II is a neutral compound, soluble only in concentrated hydrochloric or sulfuric acids; upon immediate dilution the eneamine is recovered unchanged. Refluxing II in either acidic or alkaline solution gives the enol (IV). As a vinylog of an acyl cyanoacetate[4] the enol is acidic enough to be titrated quantitatively to the phenolphthalein end-point.

Extensive hydrolysis of the eneamine (II) in acid solution gave 3-methyl-2-cyclohexene-1-one. This is analogous to the hydrolysis and decarboxylation of diethyl 3-oxo-2-cyclohexene-1-methylmalonate previously reported.[5]

The monoethylene ketal (I) was condensed with cyanoacetic acid in the presence of ammonium acetate[6] and there was obtained, in low yield, the ethylene ketal of 3-oxo-1-cyclohexeneacetonitrile.

A reductive condensation[7] with ethyl cyanoacetate proceeded readily to the ethylene ketal of ethyl 3-oxo-cyclohexaneacetate. Hydrolysis and decarboxylation of this ketal gave 3-oxo-cyclohexaneacetic acid.

Attempted reductive amination of the monoethylene ketal (I) gave no reproducible results.

Experimental[8]

Monoethylene Ketal of 1,3-Cyclohexanedione.—The preparation as described previously[1] was simplified by extraction of the ketal from the benzene solution with 2 *N* sodium metabisulfite. A 35% yield of pure ketal was obtained after saturation with potassium carbonate, extraction with chloroform and distillation.

Ethyl α-Cyano-3-amino-2-cyclohexene-$\Delta^{1,\alpha}$-acetate (II).—A mixture of 31.2 g. (0.2 mole) of the monoethylene ketal of 1,3-cyclohexanedione (I) and 25 g. (0.22 mole) of ethyl cyanoacetate were placed in a citrate bottle with 75 ml. of absolute alcohol saturated at −10° with ammonia (*ca.* 15 g. of ammonia). The solution turned yellow, became warm and after about 30 min. a precipitate separated. After an hour the reaction had subsided and the solution was cooled in ice and filtered. The product was washed with water and air-dried to give 30 to 37 g. (82–90% yield) of yellow crystalline material melting at 230–233° with dec. An analytical sample was prepared by recrystallization from 95% ethanol and from dimethylformamide–water, m.p. 232–233.5° dec.

Anal. Calcd. for $C_{11}H_{14}N_2O_2$: C, 64.06; H, 6.84; N, 13.59; mol. wt., 206. Found: C, 63.97; H, 6.74; N, 13.32; mol. wt., 196.

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[1] M. W. Cronyn and J. E. Goodrich, This Journal, **74**, 3331 (1952).

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[3] A. C. Cope and E. M. Hancock, *Org. Syntheses*, **25**, 46 (1945); E. J. Cragoe, C. M. Robb and J. M. Sprague, *J. Org. Chem.*, **15**, 381 (1948).

[4] G. E. K. Branch and M. Calvin, "The Theory of Organic Chemistry," Prentice–Hall, Inc., New York, N. Y., 1941, p. 268.

[5] C. Clemo, W. Cocker and S. Hornsby, *J. Chem. Soc.*, 616 (1946).

[6] D. E. Whyte and A. C. Cope, This Journal, **65**, 1999 (1943).

[7] E. R. Alexander and A. C. Cope, *ibid.*, **66**, 886 (1944).

[8] Analyses by the Microanalytical Laboratory of the Department of Chemistry, University of California. The ultraviolet absorption spectra were taken in 95% ethanol using a Beckman model DU spectrophotometer. All melting points are corrected.