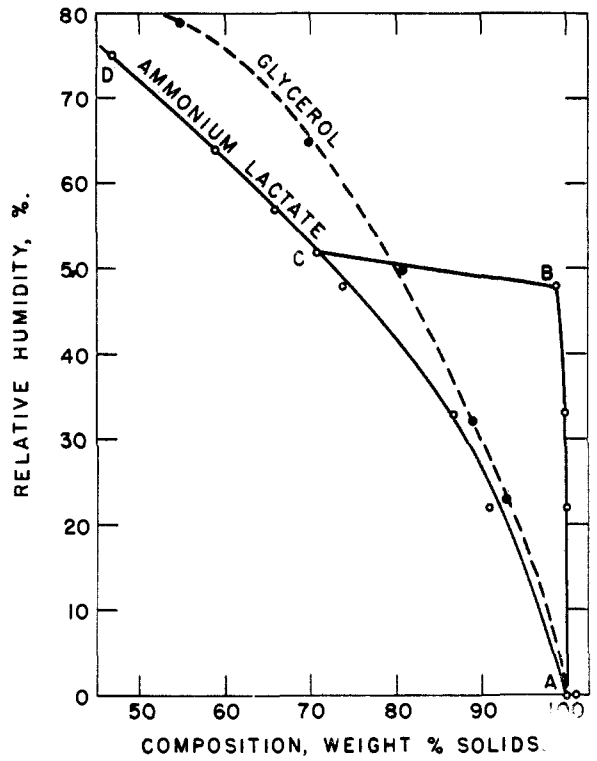

Fig. 1.—Hygroscopicity of ammonium lactate at 25°.

of zero and 33% crystallized when seeded with solid ammonium lactate. Although other equilibrium compositions were not tested in this manner, presumably all compositions between C and A of curve DCA (Fig. 1) would be supersaturated solutions of ammonium lactate.

TABLE II

HYGROSCOPICITY OF AMMONIUM LACTATE AT 25°

Relative humidity, %	Equilibrium compn., % NH_4 lactate Absorp.[a]	Desorp.[b]	Relative humidity, %	Equilibrium compn., % NH_4 lactate Absorp.[a]	Desorp.[b]
0	100	101	52	71	..
22	100	91	57	66	..
33	100	87	64	59	59
48	99	74	75	47	..

[a] Crystalline salt exposed to the various relative humidities. [b] Equilibrium compositions, obtained by absorption, transferred to atmospheres of lower relative humidity.

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An Expression for Gradient Elution

BY ARTHUR CHERKIN, FRANK E. MARTINEZ AND MAX S. DUNN

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In discussing column chromatography Tiselius[1] has said that "one of the most important practical problems of chromatography is to eliminate tailing as far as possible." One solution to the problem is gradient elution, which has been found to reduce tailing and to improve fractionation of compounds adsorbed on columns.[1-4] In such elution the concentration of elutant is increased smoothly as elution proceeds, with the effect of accelerating the tail by a higher concentration of elutant than is present at the front.

In the usual apparatus, a relatively concentrated stock solution of elutant is added dropwise to a mixing reservoir fitted with a magnetic stirrer and partly filled with a dilute solution of elutant. A side outlet near the bottom of the reservoir is joined to the top of the chromatographic column. Since the only openings in the reservoir are the inlet and outlet, inflow and outflow rates are equal.

In using gradient elution, it is helpful to be able to pre-determine the initial and final concentrations of elutant entering the column and the total volume of eluate. To this end, the relationships of the variables involved were expressed as a differential equation which upon solution gave the general expression

$$C/C_0 = (e^K - 1)/e^K$$

where C = concentration of elutant in solution leaving reservoir; C_0 = concentration of elutant in stock solution entering reservoir; K = ratio of the volume of eluate collected to the volume of diluent in the mixing reservoir.

The values of C/C_0 obtained for different values of K are plotted in Fig. 1, which emphasizes the fact that for a linear change in elutant concentration the value of K should not exceed unity. That is to

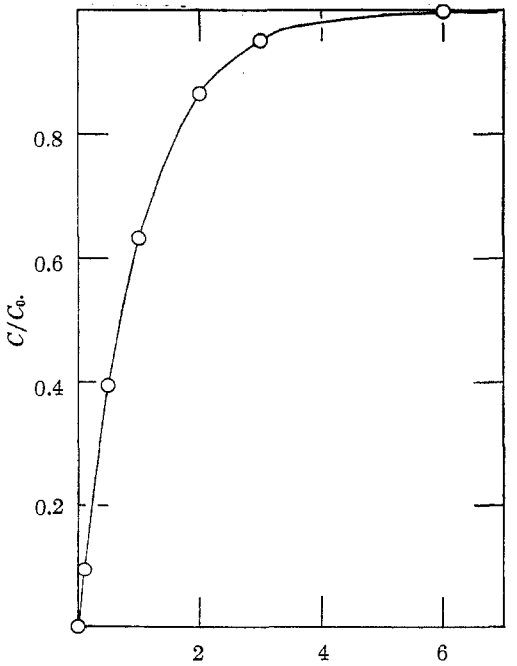

Fig. 1.—Relationship of $C/k \cdot C_0$ (ratio of concentration of elutant in solution leaving mixing reservoir to its concentration in solution entering reservoir) to K (ratio of volume of solution which has entered reservoir to volume of diluent originally placed there).

(1) A. Tiselius, *Endeavour*, **11**, 5 (1952).

(2) H. Busch, R. B. Hurlbert and V. R. Fields, *J. Biol. Chem.*, **196**, 717 (1952).

(3) K. O. Donaldson, V. J. Tulane and L. M. Marshall, *Anal. Chem.*, **24**, 185 (1952).

(4) L. Hagdahl, R. J. P. Williams and A. Tiselius, *Arkiv. Kemi*, **4**, 193 (1952).

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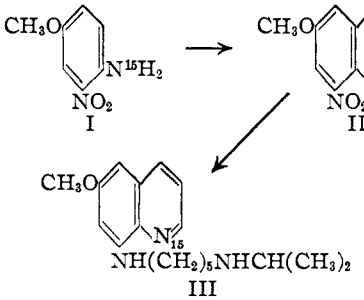
Synthesis of Pentaquine Labeled in the Quinoline Ring with N^{15} [1]

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

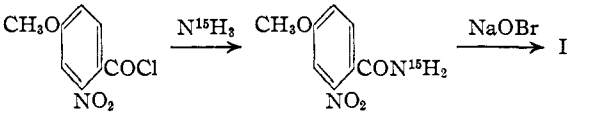
RECEIVED NOVEMBER 4, 1952

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]

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

(2) Department of Chemistry, University of Michigan, Ann Arbor, Michigan.

(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).

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(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

(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).