cell and can be used to study the action of chemical compounds on the tissue regulation of respiration.

In our investigations, as in those of Scholz et al. [3] on perfused rat liver, it has been established that the indices of the consumption of O_2, calculated to 1 mg of moist tissue per min for normal animals and for animals receiving phenobarbital, are similar. Taking into account the fact that the capacity for microsomal oxidation increases several fold in the latter case [7] and the amount of formaldehyde formed on the administration of aminopyrine also rises, it may be concluded that there is a redistribution of O_2 over different consumption pathways.

A similar redistribution also takes place under conditions of the successive limitation of respiration of sections by the concentration of O_2 in the incubation medium (see Fig. 3).

The facts presented show the desirability in testing the action on tissue respiration of any drugs that may be substrates for microsomal oxidation, of, in the first place, relating this action to the functional state of the respiratory apparatus and, in the second place, performing a determination of the effect of the drug with a change in the ratio of mitochondrial and microsomal absorption of O_2. An analysis of the kinetic characteristics of respiration can be used in evaluating the action of a substance on the various oxygen-consuming systems of the cells.

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^{14}C-LABELED DIOXIDINE

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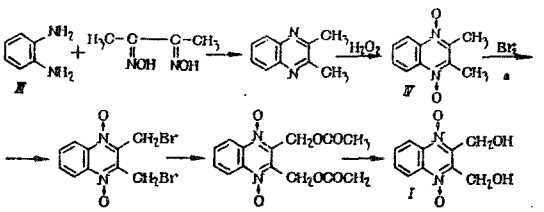
Dioxidine — 2,3-di(hydroxymethyl)quinoxaline 1,4-di-N-oxide (I) — is a new antibacterial preparation developed in the S. Ordzhonikidze All-Union Scientific-Research Institute of Pharmaceutical Chemistry. The task of the present work was to develop a method for synthesizing this drug containing a carbon-14 isotopic label. Methods of obtaining dioxine (I) by the following scheme have been described [1, 2, 3]:



A method of obtaining 2,3-dimethylquinoxaline di-N-oxide (IV) from benzofuroxan is also known [4, 5]: (see scheme on following page)

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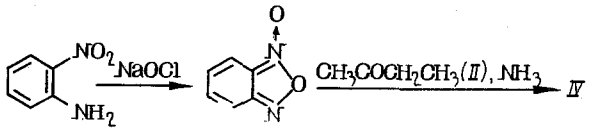


On considering these variants, it can be seen that the label can be introduced both into the benzene and into the pyrazine ring of the quinoxalin. The use of benzene labeled with ^{14}C is undesirable since, in the first place, it is expensive, in the second place, synthesis is considerably prolonged, and in the third place, the nitration of aniline forms isomers, which must sharply decrease the yield of required products.

To introduce the carbon label by the second method into the pyrazine ring it is necessary to obtain [^{14}C]butan-2-one (II), but its yield on preparation by known methods is low and the synthesis is associated with the distillation of small amounts of volatile products [6, 7].

More attractive is the first method, based on the condensation of o-phenylenediamine (III) with dimethylglyoxime, but we have not succeeded in finding in the literature descriptions of methods for introducing a carbon label into the latter reagent.

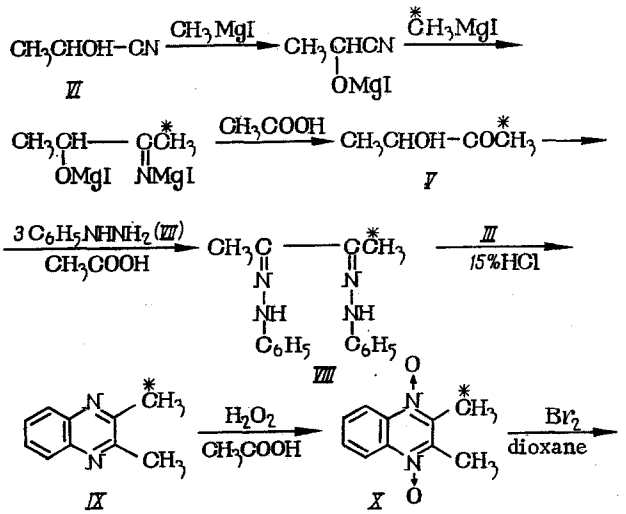
There is a report in the literature [8] on the preparation of 2,3-dimethylquinoxaline with a yield of 60% by the reaction of 2,3-dichloroquinoxaline with two equivalents of methylmagnesium iodide. We have been unable to reproduce the statements of this paper, and our yield of contaminated product did not exceed 10-15%. Attempts to obtain dimethylquinoxaline by the reaction of 3-chloro-2-methylquinoxaline with one equivalent of methylmagnesium iodide also proved to be unsuccessful.

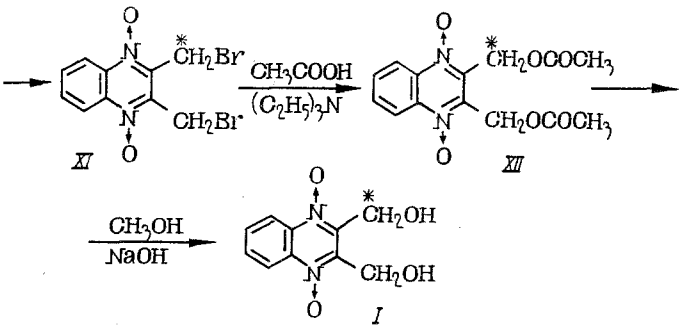
A method has been described [9] for obtaining [4-^{14}C]-3-oxobutan-2-ol ([^{14}C]acetoin) (V) with a yield of 65-75% from lactonitrile (acetaldehyde cyanohydrin) (VI) and [^{14}C]methylmagnesium iodide. Such properties of α-hydroxy carbonyl compounds, which include (V), as their capacity for readily being oxidized to α-dicarbonyl compounds and their capacity for reacting with phenylhydrazine (VIII) to form osazones, i.e., bisphenylhydrazones of α-dicarbonyl compounds, are widely known as [10].

Osazones are heavy high-melting stable and nonvolatile derivatives that are used in a number of cases for identifying α-hydroxy carbonyl compounds.

The preparation of [^{14}C]biacetyl bis(phenyl hydrazone), i.e. the osazone of [^{14}C]acetoin (VIII) without the isolation of the (V) from the reaction mixture, would make it possible to avoid the distillation of small amounts of such an unstable product. In actual fact we succeeded by this method in obtaining (VIII) and of condensing it with (III) although, it is true, under more severe conditions than in the condensation of (III) with dimethylglyoxime (15% hydrochloric acid).

The final scheme for the synthesis of [^{14}C]dioxidine had the following form:





The starting material not containing the isotopic label is the nitrile (VI) obtained from acetaldehyde and sodium cyanide. The radioactive raw material was [^{14}C]methyl iodide. The synthesis of (I) included the following stages:

1) the preparation of a solution of CH_3MgI with a known content of the Grignard reagent;

2) the preparation of an ethereal solution of $^{14}CH_3MgI$;

3) the preparation of [4-^{14}C]-3-oxobutan-2-ol (V) and its conversion without isolation into the osasone (VIII);

4) the condensation of (VIII) with (III) to form [^{14}C]dimethylquinoxaline (IX);

5) the oxidation of (IX) to [methyl-^{14}C]dimethylquinoxaline di-N-oxide (X);

6) the bromination of (X) to [methyl-^{14}C]-2,3-di(bromomethyl)quinoxaline di-N-oxide (XI);

7) the conversion of (XI) into [methyl-^{14}C]-2,3-di(acetoxymethyl)quinoxaline di-N-oxide (XII); and

8) methanolysis of (XII) with the formation of [^{14}C]dioxidine (I).

The stages from the 5th to the 8th were performed basically by published methods [1-3], with the slight variations necessary for performing the synthesis in small amounts and using an isotopic label.

EXPERIMENTAL

1. Preparation of a Solution of Inactive Methylmagnesium Iodide. A generally adopted method was used, starting from 2.4 g of powdered magnesium, 5.1 g of methyl iodide, and 50 ml of dry ether. The resulting solution was diluted with 20 ml dry ether and transferred to a 250-ml conical flask. On the following day, a sample of the solution was analyzed; the amount of CH_3MgI was 1.325 mmole in 1 ml.

2. Preparation of [^{14}C]Methylmagnesium Iodide. With stirring and spontaneous heating, a solution of 1.87 g of methyl iodide (13.4 mmole, 45 mCi, specific activity 55 mCi/ml) in 10 ml dry ether was added dropwise to 0.385 g (16 mmole) of magnesium powder and 2 ml of dry ether. The resulting solution was stored in the dark for about 1 h, after which it was used in the following stage.

3. [1-^{14}C]Butan-2,3-dione Bisphenylhydrazone (VIII). With ice-water cooling and vigorous stirring, a solution of 14.8 mmole of methylmagnesium iodide in 11.1 ml of ether was added dropwise to 1.00 g (14.1 mmole) of (VI) and 60 ml of dry ether over 30 min. After the end of the addition, the dropping funnel was replaced, and the solutions of [^{14}C]methylmagnesium iodide obtained previously, which had been transferred into a Grignard funnel with the aid of 15 ml of dry ether, was added dropwise over 15 min to the vigorously stirred reaction mixture. Stirring was continued at a gentle boil for 1 h, and then the mixture was cooled. A solution consisting of 6 g (55.5 mmole) of phenylhydrazine, 15 ml of acetic acid, and 30 ml of water was added carefully in drops with the simultaneous distillation off of the ether. After the addition, the ether was distilled off completely and the residue was boiled with a reflux condenser at 100°C for 3 h. It then was cooled, and the precipitate that had deposited was filtered off and washed with 50 ml of water and 30 ml of ethanol. After drying in the air, 2.058 g (7.72 mmole; 57.5%) of (VIII) was obtained with mp 242°C [10].†

†When the synthesis was carried out without the use of the ^{14}C isotope, the yield of (VIII) amounted to 80-85%.

4. [^{14}C]-2,3-Dimethylquinoxaline (IX). A mixture of 2.058 g (7.2 mmole) of (VIII), 1.63 g (15.42 mmole) of the base (III), 60 ml of water, and 22 ml of concentrated hydrochloric acid was boiled with periodic stirring until the solid matter had dissolved completely ($1^1/_2$ h). Then, at a temperature not exceeding 40°C, the pH of the reaction mixture was brought to 10.0 to universal indicator by the addition of 30% caustic soda solution, water was added to 300 ml, and the contents of the flask were distilled. About 250 ml of distillate was collected, and this was cooled to 0°C for 1 h and the precipitate of (IV) that had deposited from it was filtered off. Yield 0.877 g (71.6%), mp 106°C [8].

5. [^{14}C]-2,3-Dimethylquinoxaline 1,4-Di-N-oxide (X). A mixture of 0.874 g (5.52 mmole) of (IX), 8 ml of acetic acid, and 1 ml of 33% hydrogen peroxide was heated at 75-80°C and stirred for 18 h with the addition of 0.5 ml portions of hydrogen peroxide 1, 2, and $3^1/_2$ h from the beginning of heating. Then the reaction mixture was cooled and, at a temperature not exceeding 25°C, the total amount of acetic present in it was neutralized with the calculated amount of 20% caustic soda solution, for which 5.3 g of caustic soda was required. The reaction mixture was transferred to a separating funnel and was extracted with chloroform (4 × 50 ml). The solvent was distilled off from the chloroform extract in vacuum at a bath temperature not exceeding 40°C. The residue was recrystallized from 10 ml of methanol. This gave 0.827 g (4.35 mmole, 78.7%) of (X) with mp 190°C. According to the literature, mp 186-188°C (tech.) [1] and 189-190°C (after crystallization from methanol).

6. [^{14}C]-2,3-Di(bromomethyl)quinoxaline 1,4-Di-N-oxide (XI). A mixture of 5 ml of dioxane, 0.63 ml of bromine, and 0.827 g (4.35 mmole) of compound (X) was stirred at 85°C for 3 h, then was cooled to 0°C and the precipitate formed was filtered off on a porous glass filter. The precipitate was washed with 1 ml of cooled dioxane and transferred to a 25-ml beaker where the product was converted into the base by the addition of small portions of aqueous sodium bicarbonate solution. The base was filtered off, washed with water, and dried in vacuum. This gave 1.233 g (3.5 mmole; 80%) of (XI) with mp 176°C. According to the literature, mp 182.5-184°C (from benzene) [3]. The product was then converted into quinoxidine without additional purification.

7. [^{14}C]-2,3-Bis(acetoxymethyl)quinoxaline 1,4-Di-N-oxide ([^{14}C]quinoxidine) (XII). At a temperature not exceeding 25°C, 1.75 ml of acetic acid 1.43 g (2 ml) of triethylamine, 1.23 g (3.5 mmole) of (XI), and 11 ml of acetone were mixed together. Then the reaction mixture was stirred at 60-62°C (with the acetone boiling) for 3 h. After this, it was cooled to 0°C for 1 h and the precipitate that had deposited was filtered off and was washed on the filter with 2 ml of cold acetone and 10 ml of water. After drying in vacuum, 0.726 g (68%) of (XI) was obtained with mp 176°C. According to the literature, mp 176-177°C [3].

8. [^{14}C]-2,3-Bis(hydroxymethyl)quinoxaline 1,4-Di-N-oxide ([^{14}C]dioxidine) (I)†. A mixture of 0.308 g of (XII), 6.5 ml of methanol, and 0.038 ml of and 8% solution of caustic soda in methanol was stirred on the water bath at 26-27°C for 4 h. Then it was cooled to 0°C, and the precipitate was filtered off on a glass filter and was washed with 1 ml of methanol and 10 ml of chloroform. This gave 0.154 g (70%) of product with mp 171°C having a radiochemical purity determined by thin-layer chromatography† of 100%, there being no inactive spots. The specific activity was 15 mCi/g.

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†Chromatography was performed on Silufol plates in the chloroform–ethanol (19:1) system with "markers." To determine the amount of radioactive subatances, the chromatogram was counted in a counter in sections.

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