A New Synthesis of the 8-Methyl Ether of Xanthurenic Acid-Methoxy-^{14}C Employing Selective o-Methylation of Xanthurenic Acid

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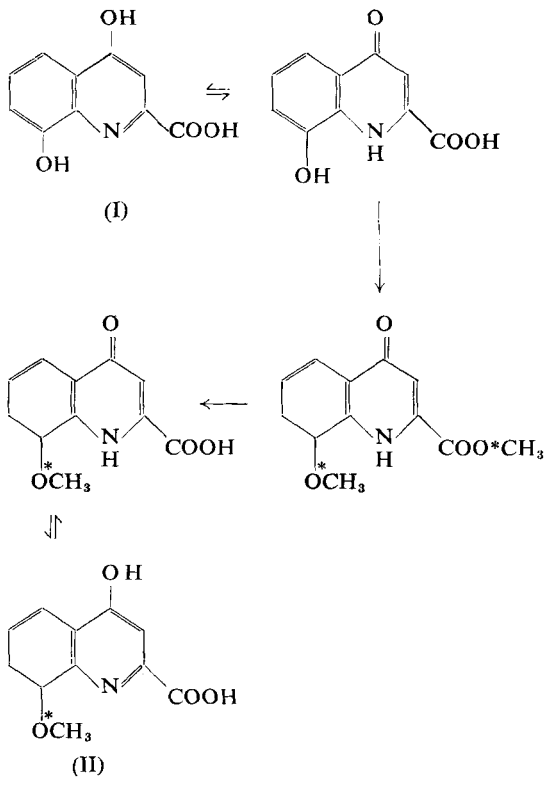
The 8-methyl ether of xanthurenic acid (II) has been identified as a urinary metabolite of the amino acid tryptophan in humans [1]. II has been shown to have carcinogenic activity for the urinary bladder [2, 3] and lymphoreticular system [4] of the mouse, and may play a causal role in the production of spontaneous human tumors. Although methods were available [5] which could be applied to the synthesis of II-4-^{14}C, it was particularly pertinent to study the metabolism of this compound with a ^{14}C-labeled methoxy group since this functional group may play a role in the mechanism of carcinogenesis [6].

The general synthesis of II as described by Price and Dodge [1] utilizing the method of Furst and Olson [7] was judged unsuitable because it did not permit a simple, continuous synthetic pathway beginning with commercially available radio-chemicals. However, a unique new synthesis of II allowing the insertion of a methoxy radioactive label was designed when it was discovered that the 8-hydroxyl of xanthurenic acid (I) could be selectively *o*-methylated.

See scheme on following page.

After the methylation of I with dimethyl sulfate, 10 µl of the reaction mixture were applied as spots on Eastman silica gel Chromagram type K301R (Eastman Kodak, Rochester, New York, USA) which were developed with chloroform, methanol, and glacial acetic acid (75:20:5 v/v). Under ultraviolet light, spots were visualized corresponding to I, II, and probably their respective methyl esters. The latter could be quantitatively converted to the free acids by alkaline hydrolysis giving a reaction mixture containing only I and II. This phenomenon can probably be attributed to the predominance of the keto tautomer of I in alkaline solution. I and II were efficiently separated on a paper powder column using a modified Mason-Berg solvent system [8]. Yields of II ranged from 50 to 60 %. The specific activity of the II-methoxy-^{14}C was below the theoretical level due to a rapid exchange between dimethyl sulfate-^{14}C and methanol. However, solvents such as acetone, ether, ethyl acetate, dimethyl sulfoxide and dimethyl formamide were not applicable due to the low solubility of I. Water and alcohols such as ethanol and propanol also exchanged with dimethyl sulfate and gave lower yields than when methanol was used. Specific activity could be increased by using anhydrous conditions



and a minimum volume of methanol. It was also noticed that yields of II were somewhat lower when using dimethyl sulfate-^{14}C than when using unlabeled dimethyl sulfate.

Other methods for the preparation of 8-alkoxy quinolines, utilizing modifications of the Skraup synthesis [9, 10] or the fusion of 8-hydroxyquinoline with KOH prior to alkyl-bromide treatment [11], are often difficult to control or give low yields. However, the present method, in addition to being selective, ran smoothly, involved a minimum of time and expense, and gave acceptable yields of II-methoxy-^{14}C.

Experimental

Preparation of the 8-methyl ether of xanthurenic acid-methoxy-^{14}C (II).

100 mg powdered NaOH and 205 mg I (1 mM) (Abbott Laboratories, North Chicago, Illinois, USA) were added to 10 ml absolute methanol in a 2-neck round bottomed flask equipped with a rubber-stoppered injection port. This deep yellow solution was refluxed on a steam bath with the aid of

a carborundum boiling stone. To the refluxing solution was added 445 mg of unlabeled dimethyl sulfate and 59 mg (4 mM total) (1 mC) dimethyl sulfate-^{14}C (New England Nuclear, Boston, Massachusetts, USA) via the injection port. Reflux was continued for 1 hour after which time the solution had become almost colorless. The methanol was removed by distillation and the resulting residue was reconstituted with 10 ml aqueous 2.5 N NaHO. After 2 hours refluxing over a gentle flame, the reaction mixture was cooled in an ice bath and acidified with dropwise additions of 12 N HCl. The resulting crystals were collected in a 15 ml sintered glass funnel, washed with 25 ml distilled water, and dried *in vacuo* over P_2O_5.

Purification of the 8-methyl ether of xanthurenic acid-methoxy-^{14}C (II).

The crystals were eluted from the glass funnel with 25 ml solvent consisting of methanol, butanol, benzene and water (2:1:1:1 v/v) [8] to which was added 1 ml 28 % ammonium hydroxide per 100 ml solvent. This solvent was known to separate I and II on paper in an ascending system [1]. The product in this solvent was applied to a paper powder column (40 × 10 cm) and the column was developed with the same solvent. The yellow color of I and the bright blue fluorescence of II under ultraviolet light allowed visualization of the separation. The II fraction was collected and taken to dryness at 50° C on a rotary evaporator. The residue was taken up in 10 ml 2.5 N NaOH and treated with 0.5 g of decolorizing charcoal. The charcoal was removed by vacuum filtration and the filtrate was acidified with dropwise additions of 12 N HCl. The pale yellow crystals were collected and dried *in vacuo* over P_2O_5. The yield of II-methoxy-^{14}C was 68 mg, or 31 % based on I.

Purity.

The m.p., infrared spectra, ultraviolet spectra, extinction coefficients, and R_f values using 2 solvent systems were identical in all respects for II synthesized by the present method and II synthesized by the method of Price and Dodge [1]. Paper chromatograms showed only 1 spot corresponding to II and scans of the chromatograms showed the spot to correspond exactly with the only detectable radioactivity. The specific activity of the II-methoxy-^{14}C was 0.4 μC/mg.

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