Synthesis of 5-Carboxy-2′-deoxyuridine

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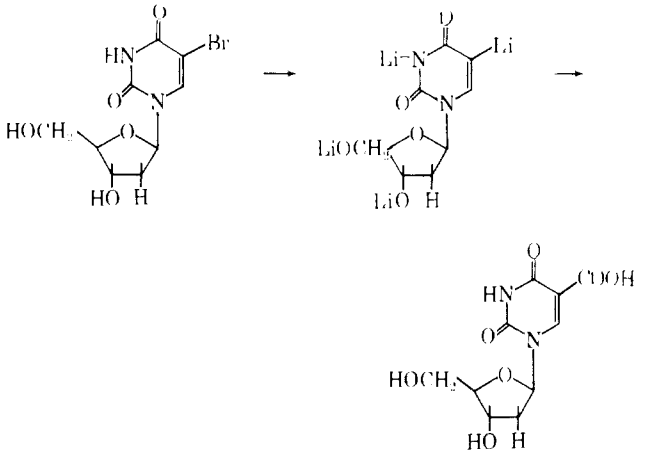
Received May 17, 1967

In view of the considerable ease with which 5-trifluoromethyluracil is hydrolyzed to 5-carboxyuracil under mild alkaline conditions,[4] it was of interest to prepare 5-carboxy-2′-deoxyuridine, which would be expected to be obtained under comparable conditions from the biologically active 5-trifluoromethyl-2′-deoxyuridine[4] and might be produced from it in the course of metabolism.[5] Furthermore, in attempts to prepare 5-trifluoromethyl-2′-deoxyuridine chemically, the possibility was considered that it might be synthesized by the SF_4 reaction[6] on 5-carboxy-2′-deoxyuridine. Attempts were made unsuccessfully to obtain the latter compound by the oxidation of thymidine by "active" manganese dioxide in analogy to the reported oxidation of thymine to 5-carboxyuracil.[7] The carboxy nucleoside was prepared from 5-bromo-2′-deoxyuridine by the reaction with butyllithium followed by carbonation (*cf.* ref 8 and 9).



The preparation of 5-carboxy-2′-deoxyuridine has been mentioned previously,[9] but the compound was obtained as a by-product and was neither isolated nor characterized. The success of the lithium reaction depends on having all reactants and equipment thoroughly dried, and carrying the reaction out in an atmosphere of pure dry nitrogen at −65°. Attempts to convert 3′,5′-diacetyl-5-carboxy-2′-deoxyuridine to 5-trifluoromethyl-2′-deoxyuridine by the reaction with SF_4 in the presence of catalytic amounts of water[6] were unsuccessful, since deoxyribose was split off even at room temperature. However, 5-carboxy-2′-deoxyuridine could also be prepared from 5-trifluoromethyl-2′-deoxyuridine by hydrolysis at pH 10.

5-Carboxy-2′-deoxyuridine at a concentration of 10^{-3} *M* failed to inhibit the growth of HeLa cells *in* culture, and hence may be considered to be biologically inert.

Experimental Section

5-Carboxy-2′-deoxyuridine.—Dry 5-bromo-2′-deoxyuridine (600 mg, 1.9 mmoles) was dissolved in 45 ml of freshly distilled tetrahydrofuran at 50° in an atmosphere of dry N_2. The solution was cooled to −65°, and 6.3 ml of butyllithium (8.5 mmoles) was injected with a syringe through a rubber diaphragm. Solid CO_2 (2 g, 45 mmoles) was added after 1 min, and the temperature was kept at −65° for 5 min and then allowed to rise gradually to 22°. The mixture was acidified with dilute HCl and then neutralized with NH_4OH. Lithium salts were removed by passing the mixture through a column of Dowex 50-H[+]. The eluate was then neutralized with NH_4OH, and the components were separated by ion-exchange chromatography on a 40 × 3 cm column of Dowex 1-formate with a linear gradient from water to 0.4 *M* formic acid. The product was eluted after 2′-deoxyuridine and 5-bromo-2′-deoxyuridine and was recovered by lyophilization to yield 107 mg of product which was crystallized from water to give colorless crystals, mp 158–159°. Since 144 mg of 5-bromo-2′-deoxyuridine was recovered, the yield, based on the bromo compound actually used, was 27%; ultraviolet spectra, in 0.1 *N* HCl λ_{max} 276 mμ (*E* 11,400), $E_{250:260}$ = 1.60; in 0.1 *N* NaOH λ_{max} 271 mμ (*E* 6810),[10] $E_{250:260}$ = 1.03; in descending paper chromatography in 2-propanol–NH_4OH–H_2O (7:1:2, v/v), R_f 0.34. Analysis by paper electrophoresis for 3 hr at 2200 v in 0.05 *M* triethylammonium bicarbonate buffer brought to pH 7.0 with formic acid gave a single spot 22.8 cm from the origin.

Anal. Calcd for $C_{10}H_{12}N_2O_7$: C, 44.12; H, 4.44; N, 10.29; neut equiv, 272.2. Found: C, 44.20; H, 4.59; N, 10.35; neut equiv, 274.

5-Carboxy-2′-deoxyuridine was also obtained when 20 mg of 5-trifluoromethyl-2′-deoxyuridine was hydrolyzed in an aqueous solution of NaOH at pH 10. The presence of the acid in the reaction mixture was confirmed by paper chromatography and ultraviolet spectrophotometry. However, only 2 mg could be isolated by ion-exchange chromatography as described above.

3′,5′-Diacetyl-5-carboxy-2′-deoxyuridine. The diacetyl derivative of 5-carboxy-2′-deoxyuridine was prepared in the usual way with acetic anhydride and pyridine,[11] and 58% was obtained, which on recrystallization from ethanol gave mp 136–138°; ultraviolet spectra, in 0.1 *N* HCl λ_{max} 275 mμ (*E* 11,500), $E_{250:260}$ = 1.62; in 0.1 *N* NaOH λ_{max} 271 mμ (*E* 7320), $E_{250:260}$ = 1.07; in the paper chromatography system described above R_f 0.46.

Anal. Calcd for $C_{14}H_{16}N_2O_9$: C, 47.19; H, 4.53; N, 7.86; neut equiv, 356. Found: C, 47.22; H, 4.65; N, 8.10; neut equiv, 359.

Reactions with Sulfur Tetrafluoride.[12] In a typical experiment, 20 mg of 3′,5′-diacetyl-5-carboxy-2′-deoxyuridine (0.059 mmoles) or of 5-carboxy-2′-deoxyuridine was treated with 20 g (0.185 mole) of SF_4 in the presence of 0.3 g of water[6] in a steel bomb for 8–9 hr at room temperature, 70–75°, and 120°. After the reaction mixture was worked up by ion-exchange chromatography, only 5-carboxyuracil and 5-trifluoromethyluracil were obtained, with more of the latter compound at the higher temperatures.

Oxidation of Thymine. Thymine (0.8 g, 6.3 mmoles) was dissolved in 500 ml of 0.1 *N* HCl, and 8 g of "active" MnO_2[13] was added. The reaction mixture was stirred for 48 hr and then worked up in the usual manner[7] to give 0.47 g (3.0 mmoles, 48%)

(1) This work was supported in part by Grants CA-7175 and CRTY-5002 from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service.

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of 5-carboxyuracil. The yield was increased to 61% when the "active" MnO_2 was washed with 15% HNO_3, followed by washing with distilled water to pH 5 (*cf.* ref 14).

Attempted Oxidation of 3',5'-Diacetylthymidine.—This compound could not be oxidized to 3',5'-diacetyl-5-carboxy-2'-deoxyuridine with "active" MnO_2, $KMnO_4$, or CrO_3.

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Preparation and Properties of Clamoxyquin Pamoate,[1] an Antiamebic and Antidiarrheal Agent

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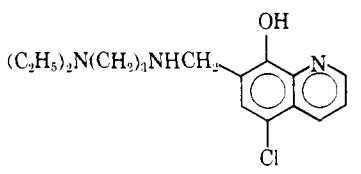
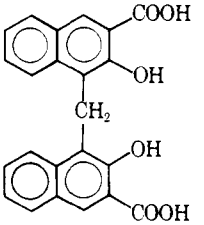
Received March 25, 1967

A wide variety of substituted 8-quinolinols has been examined in these laboratories for antiamebic and antibacterial properties.[2-4] Among them, 5-chloro-7-({[3-(diethylamino)propyl]amino}methyl)-8-quinolinol dihydrochloride (clamoxyquin hydrochloride, Ia)[3] has shown a particularly interesting degree of antiamebic activity in experimental animals.[3-5] Clamoxyquin hydrochloride formulated in gelatin capsules was



Ia, ·2HCl



b, ·pamoic acid

pamoic acid

tolerated well in man following daily 15-mg/kg doses for 5–10 days and was highly effective against various forms of intestinal amebiasis.[6,7]

Tablet and suspension formulations of clamoxyquin were of special interest for expanded clinical studies. In order to find a salt form of clamoxyquin whose taste would be more acceptable for use in aqueous suspensions for oral pediatric use, a series of salts was prepared (Table I). Among them, clamoxyquin pamoate (Ib), a salt of clamoxyquin with 1 formula weight of 4,4'-methylenebis(3-hydroxy-2-naphthoic acid), appeared to be of particular interest and was selected for extensive chemical and biological evaluation.

Clamoxyquin pamoate as the anhydrous salt contains 45.3% clamoxyquin base. The compound is relatively insoluble in water (0.004%) and is tasteless, making it well suited for use in oral pediatric suspensions.

A comparison of the antiamebic properties of clamoxyquin hydrochloride and pamoate indicated that the potent antiamebic activity of the hydrochloride salt is retained by the pamoate.[4,8] Thus, both salts were active against *Entamoeba histolytica* (200 strain) in rats when administered in the diet in dose levels of 160–679 mg/kg day for 7 days or by gavage in doses of 75–600 mg/kg day for 4 days. When dogs infected with *E. histolytica* were treated orally for 10 days, both the hydrochloride and pamoate salts were active, the cure rate being dependent upon the dosage in the range of 3.13–50 mg/kg/day and 12.5–25 mg/kg/day, respectively. These doses were well tolerated as judged by gross examination.[4]

Toxicologic studies with clamoxyquin hydrochloride and pamoate were carried out in mice, rats, dogs, and monkeys.[8,9] Acute oral LD_{50} values in albino mice were much higher for the pamoate (>2500 mg/kg) than for the hydrochloride (891.3 ± 33.7 mg/kg).[9] In chronic oral-tolerance studies in albino rats, both salts were tolerated well for 28 days at average daily drug–diet doses of 25 mg/kg; at greatly exaggerated daily doses of 200 mg/kg over the same period, the pamoate was clearly better tolerated than was the hydrochloride salt, although in neither case was evidence of organ damage observed.[9]

The two salts were also compared in subacute rising-dose-tolerance trials in dogs and monkeys.[9] Dogs tolerated up to 525 mg/kg of clamoxyquin pamoate by the end of a 35-day dosing period with manifestations similar to, but much less severe than, the gastrointestinal irritation that was seen during the administration of 250–275 mg/kg of the hydrochloride salt during 24–25 days. In monkeys, a maximum dose of 1100 mg/kg of clamoxyquin pamoate was dictated only by the bulk of drug required to deliver the dose. At this level, signs of intolerance were modest compared with those produced by similar doses of the hydrochloride salt. Therefore, in all species of animals studied to date, clamoxyquin pamoate was uniformly better tolerated than the hydrochloride salt.[9]

In definitive clinical trials, clamoxyquin pamoate formulated as compressed tablets or as a suspension was compared with iodochlorhydroxyquin[10] in the treatment of diarrheal disease.[11] Clamoxyquin pamoate was administered in a dose of 16 mg of clamoxyquin base/kg of body weight daily, in divided doses, for 5 days. Iodochlorhydroxyquin was given at the recommended dosage regimen, namely 1500 mg (500 mg tid) daily for 10 days. Clamoxyquin pamoate was tolerated well and was equal to iodochlorhydroxyquin in reducing the daily number of stools, in improving stool consistency, and eliminating fetid odor. Both drugs were effective in eliminating blood and mucus in stools. Clamoxyquin pamoate was usually better than iodochlorhydroxyquin in ameliorating subjective gastrointestinal symptoms and was superior or equal to iodochlorhydroxyquin in eliminating *E. histolytica*, *Giardia lamblia*, and *Shigella*.[7,11]

(1) Clamoxyquin pamoate is the generic name for 5-chloro-7-({[3-(diethylamino)propyl]amino}methyl)-8-quinolinol salt with 1 formula weight of 4,4,-methylenebis(3-hydroxy-2-naphthoic acid) (Clamoxyl®).

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