melting point and on sublimation experiments which were discontinued after a few milligrams of phthalic anhydride was obtained, is obviously in error. Oxidation of acridizinium bromide with potassium permanganate has now been found to afford a 36% yield of 2-(2-carboxybenzoyl)-pyridine (9).

Our nitration experiments make it clear that, in the oxidative nitration which converts the acridizinium ion into 2-(2-carboxy-4-nitrobenzoyl)pyridine (10), oxidation must precede nitration or else the product would be 3. It has now been shown that 9 is not an intermediate since it is recovered unchanged when subjected to the conditions of the oxidative nitration.

Experimental Section

10-Nitroacridizinium Perchlorate (2, X = ClO_4). To a mechanically stirred solution containing 50 ml of concentrated sulfuric acid and 50 ml of concentrated nitric acid, cooled to −5 to −10°, 5 g of acridizinium bromide[10] was added in small portions. After an additional 15 min, the mixture was poured into 400 ml of ice–water. The resulting solution was allowed to come to room temperature and filtered to remove some reddish solid. Addition of 100 ml of 35% perchloric acid to the filtrate and cooling gave 4.2 g of yellow needles. Recrystallization from water containing perchloric acid afforded 3.8 g (65.5%) of pure yellow needles, mp 197–198°.

Anal. Calcd for $C_{13}H_9ClN_2O_6$: C, 48.07; H, 2.77; N, 8.63. Found: C, 48.19; H, 2.70; N, 8.65.

The **bromide** was prepared by suspending 1 g of the perchlorate salt (2) in 25 ml of methanol which had been saturated with potassium bromide. After the slurry had been warmed and stirred for 2 hr, the potassium perchlorate was filtered off, the solution concentrated, and crystallization induced by addition of ethyl acetate. The bromide consisted of yellow platelets, mp 225–227°.

Anal. Calcd for $C_{13}H_9N_2O_2Br$: C, 48.29; H, 3.40; N, 8.67. Found: C, 48.19; H, 3.38; N, 8.79.

2-(2-Carboxy-6-nitrobenzoyl)pyridine (3). A solution of 2 g of 10-nitroacridizinium perchlorate in 20 ml of concentrated nitric acid was heated for 6 hr on a steam bath. The solution was cooled and diluted with 50 ml of water; solid sodium bicarbonate was added in small portions. At a pH ~2, 0.7 g (42%) of a colorless solid, mp 203–207°, precipitated. Recrystallization from acetic acid–water afforded a product, mp 224–225°.

Anal. Calcd for $C_{13}H_8N_2O_5$: C, 57.36; H, 2.96; N, 10.29. Found: C, 56.99; H, 3.05; N, 10.40.

2-(2-Nitrobenzoyl)pyridine (4). A mixture of 0.5 g of 3 with 0.1 g of copper powder was heated for 30 min at 180°. The solid was thoroughly extracted with boiling ethanol, and the extract filtered and concentrated, causing the crystallization of 0.25 g of tan needles, mp 117–118° (lit.[4] 117–118°).

Anal. Calcd for $C_{12}H_8N_2O_3$: C, 63.15; H, 3.53; N, 12.28. Found: C, 62.67; H, 3.52; N, 12.06.

10-Aminobenzo[*b*]quinolizidine Methiodide. A suspension of 2 g of 10-nitroacridizinium perchlorate (2) with 0.2 g of platinum oxide in 100 ml of ethanol was hydrogenated at atmospheric pressure. After absorption of the theoretical amount of hydrogen, the catalyst was removed and the solution concentrated under reduced pressure. The salt was converted into the free base from which the methiodide was prepared. The tan solid was recrystallized from methanol–ethyl acetate to give 0.7 g (32%) of tan needles, mp 254–256°.

Anal. Calcd for $C_{14}H_{21}IN_2 \cdot \frac{1}{2}H_2O$: C, 47.60; H, 6.28; N, 7.93. Found: C, 47.94; H, 6.13; N, 7.72.

8-Nitro-6,11-dihydroacridizinium Perchlorate (8). A mixture of 15 ml of concentrated sulfuric acid and 15 ml of concentrated nitric acid was cooled to 0° and 1.5 g of 6,11-dihydroacridizinium bromide (7)[8] was added over a period of 15 min. After an additional 1 hr, the mixture was poured into 200 ml of ice–water. The solution was filtered and 25 ml of 35% perchloric acid added to the filtrate. The mixture was refrigerated overnight and then collected and recrystallized from water containing a few drops of perchloric acid. The yield was 1.3 g (70%) of tan needles, mp 190–193°.

Anal. Calcd for $C_{13}H_{11}ClN_2O_6$: C, 47.79; H, 3.39; N, 8.58. Found: C, 47.70; H, 3.43; N, 8.48.

2-(2-Carboxy-4-nitrobenzoyl)pyridine (10). A. By Oxidation with Nitric Acid. A 1-g sample of 8-nitro-6,11-dihydroacridizinium perchlorate (8) in 25 ml of concentrated nitric acid was heated overnight on a steam bath. The nitric acid was removed under reduced pressure and the residue extracted with sodium bicarbonate solution. The extract was filtered and acidified, affording the crude product. Recrystallization from acetic acid–water afforded 0.3 g (36%) of product, mp 215–217°.

B. By Oxidation with Permanganate. A 1-g sample of 8 was suspended in 25 ml of hot water on a steam bath and the solution stirred while powdered potassium permanganate was added. When the color was no longer immediately discharged, the hot solution was filtered to remove manganese dioxide and then acidified. Upon cooling, 0.3 g (36%) of product, mp 217–218°, crystallized. Both products were shown to be identical by mixture melting point and ir spectra and were shown to be identical with an authentic specimen.[4]

2-(2-Carboxybenzoyl)pyridine (9). A 1-g sample of acridizinium bromide was oxidized with permanganate essentially as in the case of 8 except that acidification of the alkaline solution with dilute hydrochloric acid was carefully done to pH 5. The product consisted of colorless needles, mp 225–227°; yield, 0.3 g (36%).

Anal. Calcd for $C_{13}H_9NO_3$: C, 68.72; H, 3.97; N, 6.17. Found: C, 68.54; H, 3.87; N, 6.13.

A sample of the keto acid (9), dissolved in concentrated nitric acid and heated on the steam bath for 12 hr, was recovered unchanged.

Registry No.—1 (X = Br), 7547-88-8; 2 (X = ClO_4), 50585-79-0; 2 (X = Br), 50585-80-3; 3, 50678-82-5; 4, 50678-83-6; 7 (X = Br), 15757-24-1; 8 (X = ClO_4), 50585-82-5; 9, 27693-49-8; 10, 50585-83-6; 10-aminobenzo[*b*]quinolizidine methiodide, 50678-84-7.

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(1) This research was supported by Public Health Service Research Grant No. HE-02170 of the National Heart Institute and Grant No. CA-05509 of the National Cancer Institute of the National Institutes of Health.
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Biological Probes. I. Carbon-6-Labeled Nicotinamide

T. A. Bryson,* J. C. Wisowaty, R. B. Dunlap, R. R. Fisher, and P. D. Ellis

Department of Chemistry, University of South Carolina, Columbia, South Carolina 29208

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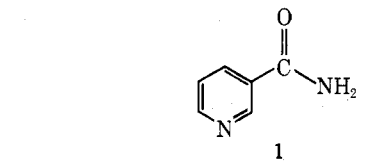
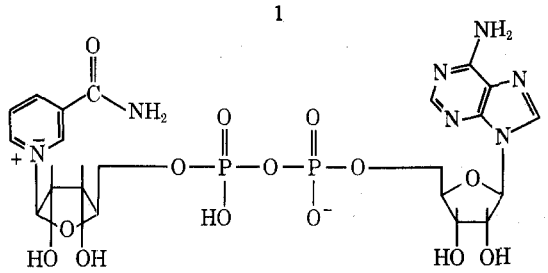
Recent interest in studies using nonradioactive labels for tracing metabolic pathways and as general biological probes has led us to develop new methods for labeling the internal ring positions of nicotinamide (1). The following describes an efficient method for preparing gram quantities of specifically labeled 1 containing ^{13}C at the C-6 position. The incorporation of this base into the coenzyme NAD^+ (2) using biosynthetic techniques can be and has been readily accomplished.[1]

The ease with which quinolines can be prepared and then oxidized to pyridinecarboxylic acids suggested that an attractive synthetic scheme could be developed incorporating these transformations as key steps. Specifically, a high yield of 2-methylquinoline-2-^{13}C (3) was obtained from *o*-aminobenzaldehyde and acetone-2-^{13}C under Friedlander reaction conditions, as is shown in Scheme I. Preliminary experiments had shown that the attempted direct oxidative degradation of 3 to a pyridinecarboxylic acid under a variety of conditions resulted in only partial



1



2

functionalization of the 2-methyl group. This problem was circumvented by transforming[2] 3 into its tribromomethyl derivative 4 prior to oxidation. Degradation of the benzenoid ring under vigorous oxidative conditions[3] led to pyridine-2,5-dicarboxylic acid, which was converted without isolation to its dimethyl ester 5. Partial hydrolysis of diester 5, according to the procedure of Isagawa and coworkers,[4] gave the monoacid 6, which was readily decarboxylated on heating in anisole to methyl nicotinate-6-^{13}C (7) in excellent yield. Nicotinamide-6-^{13}C (8) was prepared from ester 7 following standard procedures.[5] An overall yield, based on acetone-2-^{13}C, of approximately 50% for the multistep sequence can be obtained if the acidic residues of the hydrolysis and decarboxylation steps (5 → 7) were treated with diazomethane (presumably forming 5) and recycled as described in the Experimental Section. It should be noted, however, that the overall yield of labeled 1 was improved by only a few per cent by this recycling procedure. Other schemes for degrading quinoline and synthesizing pyridines to label C-6 of compound 1 were investigated prior to the above-cited labeling scheme. However, the method described herein was the method of choice owing to the high yields and reliability of each reaction. In addition, this series of reactions was designed so that each intermediate synthon that required purification was readily isolated by partitioning into the organic phase of a water–organic solvent extraction.[6]

Obviously this nonradioactive label is only of use as a biological probe or tracer agent by virtue of its spectroscopic properties, and accordingly the various nmr parameters such as chemical shifts (^{13}C, ^{1}H) and coupling constants (^{13}C–H) have been recorded in the Experimental Section. It is further evident that this reaction sequence could be adapted to label other internal ring positions of niacinamide (1). Studies are now underway to develop new techniques to specifically label additional positions of pyridine dinucleotide 2 as well as other biologically important compounds.

Experimental Section

2-Methylquinoline-2-^{13}C. To a stirred solution of sodium ethoxide (0.9 g of sodium in 100 ml of absolute ethanol) was added dropwise a solution of acetone-2-^{13}C (1.17 g, 19.8 mmol, 90% ^{13}C) with *o*-aminobenzaldehyde (2.44 g, 20.2 mmol) in 50 ml of absolute ethanol. The mixture was heated under reflux for 12 hr. The volume was reduced to 50 ml and the mixture was cautiously added to cold distilled water. After adjusting the pH to 7, the aqueous mixture was extracted with five 50-ml portions of methylene chloride. These organic extracts were combined, washed with water, and dried ($MgSO_4$). Evaporation of the volatiles afforded a residue that was distilled [bulb to bulb, 130–140° (18 mm)], giving 2.79 g (98%) of a colorless liquid: ir (neat) 3075, 1605, 820 cm^{-1}; pmr (neat) δ 2.60 (d, 3 H, $^{2}J_{C,H}$ = 6 Hz, CH_3), 6.81 (dd, 1 H, $J_{3,4}$ = 8.5, $^{2}J_{C,H}$ = 3.0 Hz, C_3H), 7.08–8.39 (m, 5 H).

2-Tribromomethylquinoline-2-^{13}C. To a mechanically stirred solution of 2-methylquinoline-2-^{13}C (2.79 g, 19.4 mmol), anhydrous sodium acetate (10 g), and glacial acetic acid (20 g) was added bromine (9.6 g, 0.12 g-atom) over a period of 15 min.

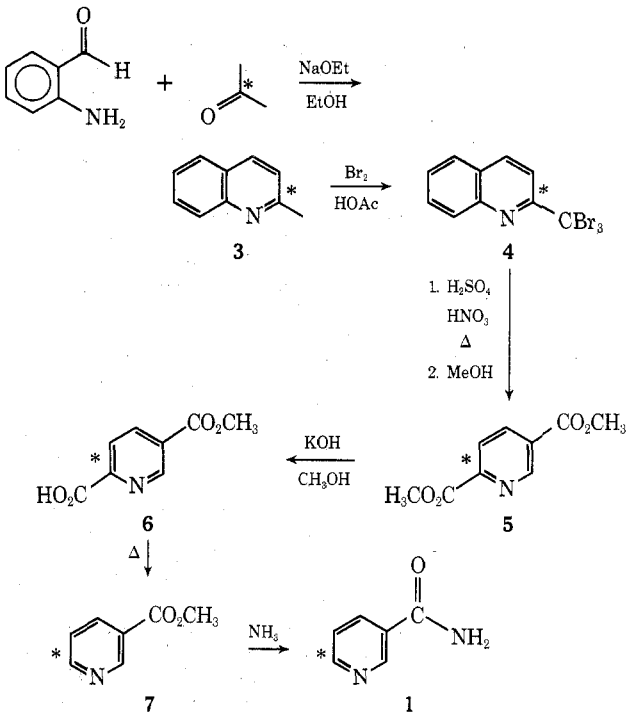
The mixture was heated at reflux for a period of 1 hr, and then cooled and poured into ice and water. The precipitate of tribromide was filtered, washed with cold water, and dried. Methylene chloride extraction (5 × 50 ml) of the combined filtrate and washing afford additional solid material upon evaporation. This material, when combined with the precipitate, afforded 6.74 g (91%) of a yellow solid (2-tribromomethylquinoline-2-^{13}C): mp 129–130° (lit.[2] mp 128°); ir (KBr) 760, 710 cm^{-1}; pmr ($CDCl_3$) δ 7.25–820 (m).

Dimethyl Pyridine-2,5-dicarboxylate-2-^{13}C. 2-Tribromomethylquinoline-2-^{13}C (6.74 g, 17.7 mmol) was dissolved in 11 ml of cold, concentrated sulfuric acid and heated with stirring until the vigorous evolution of bromine was observed (bath temperature approximately 140–150°). A slow stream of argon was passed over the mixture and the temperature was maintained at 150°. Bromine vapors were displaced by a continuous flow of argon. The temperature was then raised to 260° and concentrated nitric acid (11 ml) was cautiously added over a 2-hr period. Following addition, the excess nitric acid was removed by the use of a slow stream of argon. During this time the temperature rose to 275° and the solution became clear, bright yellow in color.

The mixture was cooled to room temperature and slowly added to cold methanol (200 ml). The methanolic solution was maintained at reflux for 18 hr. The volume was slowly reduced to 50 ml by periodic distillation of methanol and the resulting solution was poured onto ice and neutralized with sodium bicarbonate. The aqueous mixture was extracted five times with 125-ml portions of methylene chloride. The combined organic extracts were washed with water and dried ($MgSO_4$). Concentration of the organic solution (*in vacuo*) left 2.46 g (71%) of diester: mp 166° (lit. mp 164°); ir (CH_2Cl_2) 1730, 1270, 1130 cm^{-1}; pmr ($CDCl_3$) δ 3.98 (s, 3 H, CH_3), 4.03 (s, 3 H, CH_3), 8.14 (ddd, 1 H, $J_{3,4}$ = 8.2, $J_{3,6}$ = 0.8, $^{2}J_{C,H}$ = 1.5 Hz, C_3H), 8.42 (ddd, 1 H, $J_{3,4}$ = 8.2, $J_{4,6}$ = 2.0, $^{3}J_{C,H}$ = 7.0 Hz, C_4H), 9.25 (ddd, 1 H, $J_{3,6}$ = 0.8, $J_{4,6}$ = 2.0, $^{3}J_{C,H}$ = 11.5 Hz, C_6H); cmr (CH_2Cl_2) 151.5 ppm.

5-Carbomethoxypyridine-2-carboxylic Acid-2-^{13}C. Dimethyl pyridine-2,5-dicarboxylate-2-^{13}C (1.58 g, 81 mmol) was dissolved in 50 ml of absolute methanol and a solution of 85% potassium hydroxide (0.54 g, 82 mmol) in 15 ml of absolute methanol was added. The stirred solution was maintained at reflux for 2 hr and the solvent was removed under reduced pressure. The residue was dissolved in water and acidified to pH 2, extracted with methylene chloride (150 ml), and then subjected to continuous (methylene chloride) extraction for 48 hr. The organic extracts (continuous and separative) were combined, dried ($MgSO_4$), and concentrated under reduced pressure to give monoacid ester (1.29 g,

Scheme I



88%) as a white solid: mp 186–187° (lit.[4] mp 186°); ir (KBr) 1700, 1280 cm^{-1}; pmr (CF_3CO_2H) δ 4.22 (s, 3 H, CH_3), 8.76–9.70 (m, 3 H, C_3H, C_4H, and C_6H).

Methyl Nicotinate-6-^{13}C. A suspension of 5-carbomethoxypyridine-2-carboxylic acid-2-^{13}C (2.05 g, 11.3 mmol) in anisole (50 ml) was refluxed for 6 hr. The anisole solution was concentrated to 5 ml by fractional distillation (at atmospheric pressure). This mixture was then distilled at 14 mm (bulb to bulb) at 100–140°, giving a clear, colorless solution of methyl nicotinate-6-^{13}C in anisole.

The distillation residue (0.25 g) was dissolved in methylene chloride and treated with excess diazomethane. The dimethyl pyridine-2,5-dicarboxylate thus obtained could successfully be recycled (hydrolysis and decarboxylation), yielding additional quantities of methyl nicotinate-2-^{13}C.

The spectral characteristics of the anisole solution of methyl nicotinate-6-^{13}C were ir (anisole) 1725 and 1120 cm^{-1}; pmr (anisole) δ 3.57 (s, 3 H, CH_3), 6.4–7.2 (m, anisole ring protons and C_5H), 7.95 (dddd, 1 H, $J_{2,4}$ = 2.2, $J_{4,5}$ = 8.0, $J_{4,6}$ = 1.8, $^3J_{C,H}$ = 7.5 Hz, C_4H), 8.47 (ddd, 1 H, $J_{4,6}$ = 1.8, $J_{5,6}$ = 5.0, $^1J_{C,H}$ = 180 Hz), 9.14 (dd, 1 H, $^3J_{C,H}$ = 11.5, $J_{2,4}$ = 2.2 Hz, C_2H); cmr (anisole) 153.57 ppm.

Nicotinamide-6-^{13}C. The combined portions of methyl nicotinate in anisole were mixed with 150 ml of water and cooled in an ice bath. Ammonia was bubbled through the mixture for 6 hr at 3° and then for 12 hr at room temperature. Ether (50 ml) was added and the layers were separated. The etheral solution was extracted with water (25 ml). The combined aqueous portions were lyophilized, leaving 1.17 g (85%)[7] of a white solid: mp 128–130° (lit. mp 129.5–130.5°); ir (KBr) 3350, 3160, 1675, 1395 cm^{-1}; pmr (D_2O) δ 7.50 (m, 1 H, $^2J_{C,H}$ = 3.5, $J_{4,5}$ = 8.2, $J_{5,6}$ = 5.1 Hz, C_5H), 8.13 (m, 1 H, $^3J_{C,H}$ = 7, $J_{2,4}$ = 2.2, $J_{4,6}$ = 1.8, $J_{4,5}$ = 8.2 Hz, C_4H), 8.61 (ddd, 1 H, $^1J_{C,H}$ = 182, $J_{4,6}$ = 1.8, $J_{5,6}$ = 5.1 Hz, C_6H), 8.78 (dd, 1 H, $^3J_{C,H}$ = 11, $J_{2,4}$ = 2.2 Hz); cmr (D_2O) 152.64 ppm.

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Registry No.—1, 50790-51-7; 3, 50790-52-8; 4, 50790-53-9; 5, 50790-54-0; 6, 50790-55-1; 7, 50790-56-2; o-aminobenzaldehyde, 529-23-7; acetone-2-^{13}C, 3881-06-9.

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(7) Based on per cent conversion of 5-carbomethoxypyridine-2-carboxylic acid-2-^{13}C.

Chlorination of Phenols with Chlorine and *tert*-Butyl Hypochlorite. A Comparison

William David Watson

Organic Chemicals Production Research, The Dow Chemical Company, Midland, Michigan 48640

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In 1971, a review was written by Pearson and Buehler[1] on unusual electrophilic aromatic substitution reactions. A section discusses ortho enhancement, which was defined as a reaction which produces more than 66.7% ortho substitution, the statistical percentage of this position. The review states that the first successful attempts in the ortho chlorination of phenols were accomplished by *tert*-butyl hypochlorite usually in ethanol and/or carbon tetrachloride. The review also states that solvent effects and temperature have a slight influence on the ortho/para ratio but not to the extent that one would choose these factors alone to control orientation.

The statement concerning *tert*-butyl hypochlorite is derived from the work of Clark[2,3] and Ginsburg.[4,5] Clark[2,3] reported that the *tert*-butyl hypochlorite chlorination of phenol in ethanol yields 92.3% 2-chlorophenol and 87.4% 2,4-dichlorophenol, while in carbon tetrachloride it yields 57% 2-chlorophenol. Ginsburg[4,5] reported that *tert*-butyl hypochlorite reacts with 2-chlorophenol in carbon tetrachloride to yield 73% 2,6-dichlorophenol, a result which is quoted in a text on aromatic halogenation,[6] but reacts with 2-methylphenol to yield only 31% 6-chloro-2-methylphenol. Ginsburg[5] implied that alkyl hypochlorites attack phenol by a free-radical mechanism.

In 1961, Harvey and Norman[7] investigated the chlorination of various aromatic compounds, including phenols, with chlorine and *tert*-butyl hypochlorite utilizing vpc for analysis. Chlorine chlorination of molten phenol yields 39.5% 2-chlorophenol and 60.5% 4-chlorophenol. These results have been substantiated by Bing[8] and Zee.[9] Chlorination of phenol with chlorine in carbon tetrachloride yielded 74.0% 2-chlorophenol and with *tert*-butyl hypochlorite 51.0% 2-chlorophenol. From these data they concluded that *tert*-butyl hypochlorite does not give rise to high ortho/para ratios in the chlorination of phenols. Based on their observations, Harvey and Norman also proposed and gave evidence that the reaction was ionic *via* the formation of chlorine rather than free radical.

Harvey and Norman, however, did not repeat the work of Clark[2] or Ginsburg.[4] All of these authors ran their reactions at various concentrations and temperatures. For example, Harvey and Norman[7] chlorinated a 5.3% solution of phenol in carbon tetrachloride with chlorine and a 13% solution of phenol in carbon tetrachloride with *tert*-butyl hypochlorite.

One of the purposes of this work is to reexamine the chlorination of phenol in ethanol with *tert*-butyl hypochlorite. Temperature and concentration effects on the chlorination of phenol in carbon tetrachloride with chlorine and *tert*-butyl hypochlorite will be reported. Finally the results of a direct comparison of the chlorination of three phenols (phenol, 2-methylphenol, and 2-chlorophenol) with chlorine and *tert*-butyl hypochlorite, maintaining a constant temperature and concentration, will be discussed.

Results and Discussion

The claim of Clark[2] that chlorination of phenol with *tert*-butyl hypochlorite in ethanol yields 92.3% 2-chlorophenol could not be reproduced. On the contrary, *tert*-butyl hypochlorite chlorination of a 10% solution of phenol in ethanol at 15° yields 30.3% 2-chlorophenol and 65.3% 4-chlorophenol for an ortho/para ratio of 0.46. Chlorination under the same conditions except that the solvent was 95% ethanol and 5% water gave essentially the same results; *i.e.*, the maximum yield of 2-chlorophenol is 29.4% and the ortho/para ratio is 0.45. However, the addition of 2 equiv of *tert*-butyl hypochlorite to a 10% solution of phenol in ethanol at 15° did yield 86.9% 2,4-dichlorophenol, in good agreement with the 87.4% yield reported by Clark.[2]

Ethanol has been reported by Campbell and Shields[10] to be one of the solvents that favor para substitution in the chlorine chlorination of 2-methylphenol. We confirmed this report by chlorinating a 10% solution of phe-