elsewhere[8] the inhibition of catechol-O-methyl transferase with pyrogallol *in vivo* produced no accumulation of endogenous norepinephrine in the brain or heart of the rat.

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# Improved preparation of 5-bromo-2′-deoxycytidine

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THE need for a large supply of 5-bromo 2′-deoxycytidine, as well as small amounts of it with suitable isotopic labels, for study in this laboratory as either a chemotherapeutic agent or one to reduce the threshold to radiation injury, has prompted us to re-examine the preparation of this compound. The method described by Frisch and Visser[1] appeared to be impractical for several reasons: first, the method involves the use of a special source of ultraviolet light and, therefore, quartz reaction vessels; second, glacial acetic acid as the solvent for bromination leads to the undesirable acetylation of the deoxyribose moiety, a circumstance which necessitates subsequent deacetylation by methanolic ammonia; third, purification of the product by the use of a column of Amberlite IR-120 ($H^+$ form) leads to extensive cleavage of 5-bromocytosine from the deoxyribonucleoside.

We have found that bromination of 2′-deoxycytidine can be conveniently and rapidly accomplished in pyridine; the yield of 5-bromo-2′-deoxycytidine is quantitative. When the crude 5-bromo-2′-deoxycytidine hydrobromide was purified by the use of a charcoal column, an 87 per cent yield of free 5-bromo-2′-deoxycytidine was obtained. The deoxyribonucleoside appears to be completely stable on the charcoal column and no formation of 5-bromocytosine was observed even after prolonged standing.

In one experiment, formamide was employed as the solvent for bromination, in a manner similar to that of an unpublished procedure of Markham, referred to by Bessman *et al.*[2], for the preparation of 5-bromo-2′-deoxycytidine triphosphate; the 5-bromo-2′-deoxycytidine thus formed was purified according to the method of Frisch and Visser.[1] As mentioned above, some cleavage of the deoxyribonucleoside was observed under these conditions; accordingly, the products were further purified with the aid of a Dowex-1 formate column. The final yield was about 60 per cent.

In the preparation of 5-bromo-2′-deoxycytidine-$H^3$ from tritiated-2′-deoxycytidine on a micromole scale, the radioactive product was purified by the use of a Dowex-1 formate column. Prompt elution of the column was necessary in order to minimize cleavage of the deoxyribonucleoside. With different batches of the resin, the yield varied from 60 to 90 per cent. The loss of radioactivity, which resulted from the replacement of hydrogen by bromine in position 5, accounted for approximately one-half of the total $H^3$ of the 2′-deoxycytidine hydrochloride; this finding gives strong support to the hypothesis that the precursor was labeled exclusively in positions 5 and 6.

## EXPERIMENTAL

5-*Bromo-2′-deoxycytidine*. (A) *Bromination in pyridine*. A mixture of 2′-deoxycytidine hydrochoride[*] (2·6 g; 0·01 mole) in pyridine (600 ml) was stirred at room temperature until partial solution of the compound was obtained. Of a solution of bromine in carbon tetrachloride (1 ml of bromine diluted with carbon tetrachloride to give a volume of 10 ml), 7 ml were added and stirring was continued at room temperature until the solution became entirely clear (30 min) (on the basis of ultraviolet absorption the bromination was nearly quantitative). The pyridine solution was concentrated at 50° *in vacuo* to dryness. The residue thus obtained was dissolved in water (70 ml) and the reaction was adjusted to pH 9 with a solution of sodium hydroxide (10 N). The solution was then put on a charcoal column[†], 2·5 × 27 cm; this was washed with distilled water until free of bromide (7–10 l.). The product was eluted from the column with 95 per cent ethanol (6–8 l.) and the solvent was removed *in vacuo* at 50°. The residue crystallized to give 5-bromo-2′-deoxycytidine in over-all yield of about 87 per cent; m.p. 171–175° (reported 175–179°[1]); $\lambda_{max}^{pH2}$ 299–300mμ (ε 9,600); $\lambda_{max}^{H_2O}$ 287 mμ (ε 5,880); $\lambda_{max}^{95\%\,EtOH}$ 290 mμ (ε 5,650); the product may be recrystallized from methanol and ethyl acetate. (B) *Bromination in formamide*. To a solution of 2′-deoxycytidine hydrochloride (1·3 g, 0·005 mole) in formamide (8 ml), a solution of bromine in carbon tetrachloride (4·4 ml; 1 ml of bromine diluted with carbon tetrachloride to give a volume of 10 ml) was added until there was a distinct light yellow color present due to a slight excess of bromine. Completion of bromination was indicated by the ultraviolet absorption spectrum of the reaction mixture, which should have a $\lambda_{max}^{pH2}$ 299 mμ. The mixture was now taken up in water (200 ml), and purified as described in (A). A less pure product can be obtained by substituting, in place of charcoal, acidic resins such as Amberlite IR-120, according to the method of Frisch and Visser[1]; with this procedure the yield of the crude 5-bromo-2′-deoxycytidine was 60 per cent. This material was rechromatographed on a Dowex-1 formate column to separate it from unreacted 2′-deoxycytidine and 5-bromo-cytosine.

5-*Bromo-2′-deoxycytidine-$H^3$*. A solution of tritiated 2′-deoxycytidine[‡] in 50 per cent ethanol (0·75 ml, 0·75 mc, 0·68 μmole) was reduced to dryness at 40° and vacuum dried at room temperature for 1½ hr. To the dried residue were added a solution of non-radioactive 2′-deoxycytidine hydrochloride in pyridine (0·27 ml; 3 mg/ml, 3·08 μmoles) sufficient to bring the specific activity of the final solution to 0·75 mc/3·76 μmoles (199 μc/μmole), and a solution of bromine in carbon tetrachloride (0·25 ml of a solution of bromine in carbon tetrachloride containing 0·1 ml per 100 ml). The solution was allowed to stand for 10 min and then evaporated to dryness at 40°, followed by brief drying under vacuum to remove the pyridine. The residue was taken up with water (1 ml); the solution was adjusted to about pH 11 with 1 N sodium hydroxide, divided into two equal portions, and put on two 30 × 1 cm Dowex-1 formate columns. These were subjected to gradient elution with water (1 l.), agitated

[*] Purchased from California Corp. for Biochemical Research, Los Angeles, Cal.

[†] Composed of activated carbon, grade 20 × 40, Atlas Powder Co., Wilmington, Delaware.

[‡] Purchased from Schwarz Bio-Research, Inc., Mt. Vernon, New York, and presumed, from the method of preparation, to contain tritium only in positions 5 and 6; specific activity, 1100 μc/μmole.

magnetically, into which 0·025 N formic acid was introduced dropwise in a continuous manner. The eluate was collected in 1-ml fractions; yield, 610 μg, specific activity, 92 μc/μmole.

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# A single extraction method for the determination of both norepinephrine and serotonin in brain

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IN THE course of studies in this laboratory on the physiologic role of brain norepinephrine and serotonin and the effects of various drugs on their levels, separate extraction methods for the two amines were developed.[1, 2, 3] In these procedures the amines are extracted from brain homogenates into *n*-butanol: serotonin from an alkaline homogenate, and norepinephrine (because of its instability in alkali) from an acid homogenate. The discovery that serotonin can also be extracted into *n*-butanol from an acid homogenate has made it possible to use the unmodified norepinephrine extraction procedure of Shore and Olin[2] for the extraction of both amines.

With the single extraction procedure, it is possible to measure the amounts of each brain amine in separate aliquots of the final acid extract. Neither amine interferes with the determination of the other. Norepinephrine was determined by oxidation, at pH 5, to a highly fluorescent trihydroxyindole[2] and serotonin by its native fluorescence in 3 N hydrochloric acid.[1]

TABLE 1. RECOVERY OF SEROTONIN ADDED TO ALIQUOTS OF BRAIN HOMOGENATE

| Endogenous serotonin | Serotonin added | Total serotonin | | Recovery of total serotonin |
|---|---|---|---|---|
| | | Calculated | Found | |
| μg | μg | μg | μg | per cent |
| 1·60 | 0·80 | 2·40 | 2·45 | 102 |
| 1·72 | 0·80 | 2·52 | 2·47 | 98 |
| 1·34 | 0·80 | 2·14 | 2·01 | 94 |
| 1·92 | 0·80 | 2·72 | 2·67 | 98 |
| 1·60 | 0·80 | 2·40 | 2·28 | 95 |

When one volume of salt-saturated 0·01 N hydrochloric acid containing serotonin is shaken with ten volumes of *n*-butanol, about 85 per cent of the serotonin is extracted into the organic phase. Serotonin added to aliquots of rabbit brain homogenates was almost completely recovered (Table 1), and the recovery of norepinephrine added to the same aliquots was the same as previously reported.[2]

The amount of serotonin found in rabbit brainstem by this technique is almost identical with that obtained by the use of the alkaline extraction procedure of Bogdanski *et al.*[1] The single extraction method yielded a mean serotonin content for normal rabbit brainstem of 0·69 μg/g ± 0·05 (s.d.), as compared with 0·66 μg/g ± 0·03 (s.d.), with the alkaline extraction procedure. The serotonin and