mixed melting point with a sample prepared from *N*-acetyl-DL-aspartic anhydride and aniline. Infrared spectra showed identical bands at 5.8, 6.0, 6.25, 6.45, 6.7 μ.

Action of ammonia on N-phenylacetamidosuccinimide. *N*-Phenylacetamidosuccinimide (XVI) (0.75 g., 0.00324 mole) was treated with 4 ml. of concd. aqueous ammonia for 12 hr. The resulting solid was filtered and washed with water. Recrystallization from 80 ml. of alcohol gave 0.61 g. (81.2%) of *N*-acetyl-DL-aspartic α-amide β-anilide, m.p. 214–216°. This product gave no depression on admixture with a sample of the compound obtained from *N*-acetyl-DL-aspartic β-anilide by the mixed anhydride method. Hofmann degradation of 50 mg. of this substance with 3.5 ml. of 0.48% sodium hypochlorite solution gave 10 mg. of acetaldehyde-dinitrophenylhydrazone m.p. 147° alone and admixed with authentic material. A paper chromatographic analysis of the hydrolyzed mother liquor failed to indicate the presence of α,β-diaminopropionic acid. In second experiment 2.039 g. (0.0088 mole) of the imide (XVI) was treated with 30 ml. of concd., aqueous ammonia at room temperature for 16 hr. and finally evaporated to dryness. Sodium hypochlorite solution (23 ml., 30.83 mg. sodium hypochlorite per ml.) was added to the residue at 0° with stirring. The stirring was continued at room temperature for 40 min. and then at 80° for 15 min. The clear solution was acidified with 15 ml. of 6*N* hydrochloric acid and distilled into 2,4-dinitrophenylhydrazine hydrochloride solution until acetaldehyde 2,4-dinitrophenylhydrazone (87.6 mg.) was formed. The remaining solution was refluxed for 5 hr. and then evaporated to dryness. The residue was treated with 5 ml. of concd. hydrochloric acid. The undissolved sodium chloride was filtered off and the filtrate was evaporated and dried over sodium hydroxide. Semiquantitative paper chromatography (butanol, acetic acid, water; 4:1:5) indicated the presence of about 150 mg. of α,β-diaminopropionic acid (10%) in addition to aspartic acid.

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[CONTRIBUTION FROM THE NEUROSURGICAL SERVICE OF THE MASSACHUSETTS GENERAL HOSPITAL AND THE DEPARTMENT OF SURGERY OF HARVARD MEDICAL SCHOOL]

Synthesis of p-[Di(2-C14-chloroethyl)amino]-L-phenylalanine. A Study of Bis(β-hydroxyethylation) of Arylamines[1]

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A study is carried out of various methods for the bis(β-hydroxyethylation) of aryl amines with a view toward synthesizing C^{14}-labelled nitrogen mustards of high specific activity. The label would be in the biologically-active portion of the molecule. The synthesis of p-[di(2-C^{14}-chloroethyl)amino]-L-phenylalanine was carried out.

The assumption that the effectiveness of cytotoxic agents in the treatment of cancer is greatest if they preferentially concentrate in neoplastic tissue would seem to be a rational hypothesis. On this basis, the treatment of brain tumors by these compounds offers a distinct advantage over such therapy for neoplasms in other regions of the body. Tumors of the brain have a considerably altered permeability to many substances relative to adjacent normal areas[2] and consequently, it seems feasible to devise cancerocidal substances which, by cerebral perfusion, would concentrate selectively in the tumor. Work in this laboratory with aromatic boron compounds[3,4] has yielded information as to types of structures and substituents which aid or restrict the passage of organic compounds into the brain. Lipid solubility has been observed to be an important criterion in determining this rate and ease of penetration of the brain by such substances.[3,4]

Previous work with P^{32}-labelled triethylene thiophosphoramide (thio-TEPA) showed[5] that alkylating agent had a high lipid solubility and penetrated normal brain more readily and accumulated in higher concentration than in the corresponding neoplastic tissue. Based on our initial hypothesis, this would be considered an undesirable compound. On the other hand, p-[di(2-chloroethyl)amino]-L-phenylalanine had a low lipid solubility[6] as determined by the standard partitioning procedure.[3] From this consideration and the known high biological activity of melphalan,[7] the preparation of this compound with a C^{14}-label was undertaken to study its localization in brain and brain tumor as a function of its lipid solubility. The radioactive DL-compound with the label in the phenylalanine position of the molecule had been prepared[8] from carboxy-labelled benzoic acid. A synthesis incorporating C^{14} in high yield in the mustard group, however, would offer two important advantages: (1) a general method for preparing most nitrogen and sulfur mustards with a C^{14} label and (2) the label would be in the alkyl-

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ating portion of the molecule and consequently, its site of action could be determined. For this purpose, several different methods for synthesizing di(2-hydroxyethyl)arylamines were investigated. Such substances would be converted readily into the corresponding di(2-chloroethyl)- or di[2-(*p*-toluenesulfonoxy)ethyl]amines.

Several methods have been described in the literature relating to the formation of di(2-hydroxyethyl)arylamines. They are: (1) the condensation of the amine with ethylenechlorhydrin in refluxing aqueous suspensions of calcium carbonate,[9] (2) the reaction of a labile aromatic halogen with diethanolamine,[10] (3) reaction of an aromatic amine with ethylene oxide in sealed tubes with or without an inert solvent,[11,12] and (4) the acid-catalyzed reaction of ethylene oxide with the amine in an aqueous solution.[9,13-15] The first method even at these high temperatures resulted in mixtures of mono- and dialkylated amines. Consequently based on the alkylating agent the yield of the di(2-hydroxyethyl)amine was low. The second procedure required an aromatic halogen activated by electron-withdrawing groups on the aromatic nucleus. Such an approach is limited to certain specific compounds and is obviously not one of general utility. The third method required high temperatures and pressures together with long reaction times and even under these forcing conditions, appreciable amounts of the monohydroxyethylated product occurred. Only by use of the fourth procedure did the preparation of an optically-active amino acid containing a C^{14} label in the mustard group appear feasible as well as a general synthetic scheme. However, even in this approach, previous workers had added a large excess of ethylene oxide to an aqueous acetic acid solution of amine. Of course, such conditions could not be used if one were to attain a good utilization of ethylene-1,2-C^{14} oxide and consequently a high specific activity of the nitrogen mustard. This necessitated an investigation of several possible methods and conditions in which the hydroxyethylating agent is the limiting component.

For the purpose of the study, ethyl *p*-aminobenzoate was used as a model compound and attempts were made to oxyethylate the amine under a variety of conditions and with different agents. The only substances which were isolated and characterized were the unchanged starting material and ethyl *p*-[di(2-hydroxyethyl)amino]benzoate. The latter was recrystallized to a constant melting point, 71°–73°, and its yields with respect to the utilization of the hydroxyethylating agent are recorded in Table I. No attempts were made to determine the amount of the monohydroxyethylamino compound present in the reaction mixture since, for the purpose of this study, the bis compound was the only one of interest.

In one series of reactions, alkylation with ethylene oxide in different molar amounts was carried out in formic, acetic, and propionic acids with varying percentages of water. Hydroxyethylation of the model amine with ethylene oxide was also attempted under anhydrous conditions in ethanol, diethyleneglycol dimethyl ether, benzene, and dioxane with and without acidic catalysts such as boron trifluoride, acetic, and *p*-toluenesulfonic acids. Basic solvents and catalysts were avoided in this study since their utilization was negated by the need for preparing a nitrogen mustard containing an optically-active amino acid.

In addition to ethylene oxide as the hydroxyethylating agent, ethylene carbonate was also used. This reagent has proved useful in the hydroxyethylation of phenols,[16,17] thiophenols, alcohols, thiols, carboxylic acids and amines.[18] The possibility of using C^{14} labelled ethylene carbonate would offer two distinct advantages: (1) its ready synthesis from ethylene-1,2-C^{14} glycol and (2) a greater ease in handling relative to low boiling ethylene oxide. As with the reactions involving ethylene oxide, only ethyl *p*-[di(2-hydroxyethyl)-amino]benzoate was isolated. In Table I the yields and conditions are recorded.

A third approach to the synthesis of bis(2-hydroxyethyl)arylamines was the attempt at catalytic reductive alkylation of the corresponding nitro compound in the presence of glycolaldehyde. This method has been utilized in the synthesis of dialkylanilines from nitrobenzene in the presence of aliphatic aldehyde.[19] However, no examples with glycolaldehyde have been reported.

DISCUSSION

As seen from Table I, the more successful incorporations of ethylene oxide occurred when the reaction was carried out in glacial acetic and propionic acids. The addition of water appreciably decreased the utilization of the alkylating agent. However when formic acid was used as the solvent only ethyl *p*-formamidobenzoate could be isolated, indicating a more rapid rate for this step relative to hydroxyethylating. Kinetic studies on the ring

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TABLE I

HYDROXYETHYLATION OF ETHYL p-AMINOBENZOATE

Solvent and Conditions[a]	% Excess of the Oxyalkylating Agent	Yield of the Ethyl di(2-Hydroxyethyl)-aminobenzoate	% Utilization of the Oxyalkylating Agent
ETHYLENE OXIDE			
50% Aqueous acetic	50	38	25
acid	100	53	27
	300	76	19
Glacial acetic acid	0	67	67
	50	80	53
	100	79	40
Propionic acid	0	46	46
	100	73	37
ETHYLENE CARBONATE			
Ethylene carbonate			
95°–100°	100	0	0
160°–163°	100	8	4
185°–190°	100	17	9
2-Methyl-2-butanol,			
102°	100	0	0

[a] Where temperatures are not listed, the reaction was carried out at room temperature.

opening of substituted ethylene oxides in aqueous solutions showed[20,21] that the hydrolytic cleavage of the epoxide is acid-catalyzed readily forming the glycols. On this basis, competitive reactions between water and the aromatic amine for the conjugate acid of the oxide can be expected. In the concentrated acids this competition could obviously not occur. Swain[22] observed that the ring opening reaction of iodide ion with a substituted epoxide occurred appreciably faster in glacial acetic acid relative to aqueous solutions. This, in addition to the fact that the acetate ion did not compete with iodide for the oxide, would suggest that glacial acetic acid would be preferable to an aqueous acetic acid solution as a hydroxyethylating medium. The results in Table I confirm this.

All other attempts in various nonaqueous solvents with and without acid catalysts were singularly unsuccessful. This includes use of ethanol, diethyleneglycol dimethyl ether, diglyme–acetic acid (1:1), diglyme–boron trifluoride, boron trifluoride etherate, and several of these solvents with catalytic amounts of acetic or p-toluenesulfonic acids. Also the amine hydrochloride of ethyl p-aminobenzoate failed to yield more than insignificant amounts of ethyl p-[di(2-hydroxyethyl)-amino]benzoate when treated in benzene, dioxane, or a benzene-ethanol (1:1) mixture with ethylene oxide. Reductive alkylation with glycolaldehyde did not succeed and the reactions with ethylene carbonate, as shown in Table I, gave poor yields of the bis compound.

On this basis the preparation of p-[di(2-hydroxy-C14ethyl)amino]-L-phenylalanine was carried out by incorporating C14-labelled ethylene oxide into p-amino-N-phthaloyl-L-phenylalanine ethyl ester in glacial acetic acid. The dihydroxyethyl derivative of the amine was not isolated but converted directly into the nitrogen mustard. The yield of the product isolated, based on the amine, was 43% and calculated on the utilization of C14-ethylene oxide was 41%.

This method for preparing C14-labelled nitrogen mustards with the label in the alkylating portion of the molecule would appear to offer general utility. Use of such compounds will permit correlation of the incorporation of the agent in the tumor tissue with biological effectiveness of the antimitotic compound. At the same time, further information will be available as to whether lipid solubility of an organic compound is an important criterion in determining the penetration of brain and brain tumor. Partition studies of C14-labelled melphalan in order to determine lipid solubility were carried out as previously described.[3] The coefficients for the aqueous/benzene and aqueous/chloroform partitions were 103 and 116 respectively. Values in this range indicate that such a compound has a very low lipid solubility and should be a desirable compound based on our initial hypothesis.

EXPERIMENTAL[23]

General. In all the hydroxyethylation experiments performed with ethyl p-aminobenzoate as a model compound, the only substances which were isolated and characterized were the starting material and the ethyl p-[di(2-hydroxyethyl)amino]benzoate. For reasons mentioned earlier, no attempts were made to determine the extent of formation of the monohydroxyethylated product.

Reactions with ethylene oxide. A. *In acetic acid and propionic acids.* In order to obtain comparable results 4.12 g. of ethyl p-aminobenzoate (0.025 mole) was dissolved in 25 ml. of the concentrated acid or in 50 ml. of the 50% aqueous acid. To the solution cooled to about 4° was added with shaking the chosen amount of ethylene oxide. After standing in a stoppered flask at room temperature for 24 hr., the reaction mixture was diluted with water to about 100 ml. and neutralized by saturation with solid sodium bicarbonate. The precipitates formed were collected, washed with small amounts of cold water, dried, and recrystallized from 70 ml. of benzene. The ethyl p-[di(2-hydroxyethyl)amino]benzoate (m.p., 71°–73°) was filtered after 24 hr. Yields and utilization of the oxyalkylating agent under different conditions are shown in Table I.

B. *In formic acid.* The conditions of the attempted hydroxyethylation and the processing of the reaction mixture using formic acid as a solvent were the same as described previously in section A. Even in the presence of a large excess of ethylene oxide, no ethyl p-[di(2-hydroxyethyl)-amino]benzoate was isolated. The only compound identified was ethyl p-formamidobenzoate which, after successive recrystallizations from benzene and water, melted at 150°–151°. The same compound was readily synthesized from the amine and formic acid in the absence of ethylene oxide.

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Anal. Calcd. for $C_{10}H_{11}NO_3$: N, 7.25. Found: N, 7.31.

Reactions with ethylene carbonate. A stirred mixture of 17.6 g. (0.2 mole) of ethylene carbonate and 8.25 g. (0.05 mole) of ethyl *p*-aminobenzoate was heated for 6 hr. at temperatures ranging from 95° to 190° as shown in Table I. After being cooled to room temperature the solidified melt was extracted with three 50-ml. portions of warm benzene. The combined extracts were allowed to cool to room temperature and after 24 hr., the crystallized products were filtered, washed with a small volume of benzene, and dried.

In another experiment the same proportions of the reactants were refluxed in 40 ml. of 2-methyl-2-butanol for 6 hr. The solution was evaporated to an oily residue *in vacuo* and worked up as described above.

Attempts at reductive hydroxyethylations. A solution of 1.80 g. of glycolaldehyde (0.03 mole) in 15 ml. of 95% ethanol containing 1.95 g. of ethyl *p*-nitrobenzoate with 1.0 ml. of glacial acetic acid as a condensing agent was hydrogenated at room temperature in the presence of 0.1 g. of platinum oxide catalyst. In a similar manner the reaction was attempted only using 1.65 g. of ethyl *p*-aminobenzoate instead of the nitro compound with 120 mg. of anhydrous sodium acetate as the condensing substance. In both cases only ethyl *p*-aminobenzoate could be isolated.

Preparation of p-[di(2-chloro-C¹⁴-ethyl)amino]-L-phenylalanine. *p*-Amino-*N*-phthaloyl-L-phenylalanine ethyl ester (I) prepared according to the method of Bergel and Stock[9] was used as starting material. All subsequent chemical transformations leading to the crude C^{14}-labelled phenylalanine mustard were performed in the same tared flask to avoid possible losses in material transfers.

In a 100-ml. one neck flask a solution of 3.073 g. (I) (9.1 mmoles m.p., 108°–109°) in 15 ml. of glacial acetic acid was maintained nearly at its freezing point. The long shaped ampule, containing 0.434 g. of ethylene-1,2-C^{14} oxide[24] (10 mmoles, specific activity 0.203 millicurie/mmole) in its bulb, was inserted in a one hole rubber stopper in such a position that the top of it would be submerged about 5 mm. under the surface of the acetic acid solution if the stopper were placed into the neck of the flask. Keeping the oxyalkylating agent in the bulb in solid state with liquid nitrogen the top of the ampule was opened. Then the ampule, by means of the stopper, was placed into position in the flask. The ethylene oxide was quantitatively transferred into the cold acetic acid solution by allowing the bulb to warm up slowly to room temperature. The ampule was rinsed by controlled warming and subsequent cooling of the flask. After completing this transfer, the solution was kept stoppered at room temperature for 18 hr. with occasional shaking. Then under the same conditions, the solution was treated with an additional 10 ml. of inactive ethylene oxide (0.2 mole) for 24 hr. in order to complete the hydroxyethylation. Finally, the mixture was concentrated and dried as a sirup in vacuo.

Without any further purification, the hydroxyethylated amine ester was dissolved in 50 ml. of benzene and the solution distilled until about 15 ml. of distillate was collected. The residual benzene solution was gently refluxed with 12 ml. of freshly distilled phosphorus oxychloride for 25 min. and then concentrated to an orange brown gum *in vacuo*. The gum was dissolved in 40 ml. of a benzene-ethanol mixture (1:1) and the solution evaporated to dryness at low temperatures. This procedure was repeated again to remove residual phosphorus oxychloride.

The residue obtained in the chlorination was dried over sodium hydroxide *in vacuo* and subsequently refluxed in 40 ml. of 6*N* hydrochloric acid for 3 hr. The hydrolysis mixture was cooled for several hours, filtered on a tared fritted glass funnel, and dried. The weight of the precipitated phthalic acid was determined in order to check the completeness of the hydrolysis. Then 100 ml. of a saturated sodium acetate solution was added in small portions to the cold filtrate. The precipitated crude L-phenylalanine mustard was collected, washed with a small amount of cold saturated sodium acetate solution, and dried, yielding 2.172 g. (73% based on the amount of I) of the impure compound. The crude substance was recrystallized from methanol by concentrating a 250 ml. alcoholic solution to a volume of 50 ml. 1.190 g. (43% yield) of *p*-[di(2-chloro-C^{14} ethyl)amino]-L-phenylalanine (m.p. 181°–184°, $[\alpha]_D^{25}$ +9.5 ± 0.6°, *c*, 0.93 g./100 ml. in 1*N* hydrochloric acid) having a specific activity of 0.213 mc./mmole was obtained. Based on the specific activity of the oxyalkylating agent, the utilization of ethylene-1,2-C^{14} oxide was 41%.

Distribution of C¹⁴-labelled melphalan between aqueous and lipid phases. Approximately 2 mg. of the C^{14}-labelled L-phenylalanine mustard was partitioned between 50 ml. of an aqueous potassium dihydrogen phosphate buffer (*p*H 7.18) and 50 ml. of benzene or chloroform. After separation of the two phases, several 0.05 ml. quantities of each layer were plated and counted. Distribution coefficients found are 103 for aqueous/benzene and 116 for aqueous/chloroform.

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[CONTRIBUTION FROM THE COBB CHEMICAL LABORATORY, UNIVERSITY OF VIRGINIA]

Reaction of Ammonia with an Aromatic Aldehyde in Dilute Solution

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The reaction of *p*-dimethylaminobenzaldehyde, at very low concentrations, with ammonia in methanol is similar in many respects to its reaction with primary amines to yield Schiff bases. The rate of the reaction is determined at 0° and 25° and the energy and entropy of activation calculated. The formation of a simple imine is inferred.

The action of ammonia on aromatic aldehydes is generally complex and usually results in the formation of hydrobenzamides,[1] the kinetics of which were investigated by Dobler[2] as early as 1922. By passing gaseous hydrogen chloride into a solution of hydrobenzamide in ethanol, Busch[3] was able to

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