

spectral analysis, and the results are shown in Table II.

(b) **Ceruloplasmin.** A preparative-scale incubation was conducted by adding 120 mg of 16-*O*-acetylvindoline (1a) and 180 mg of chlorpromazine to 600 mL of pH 5.5, 0.2 M sodium acetate buffer containing 3240 units of ceruloplasmin. This mixture was incubated at 37 °C, and the polar (R_f 0.1, solvent system A) metabolite was formed immediately. At 5 h, the transformation reaction was estimated to be 50% complete (TLC), and it was terminated by adjusting the pH to 9.5 with NH_4OH and extracting with ethyl acetate as before. The extract was subjected to preparative TLC with 0.5-mm layer silica gel GF₂₅₄ plates and solvent system A to obtain the polar metabolite free from contaminating 16-*O*-acetylvindoline. The presumed iminium derivative (2a) was dissolved in 20 mL of CH_3OD and reduced with NaBD_4 as described earlier, and the resulting analytically pure product (2 mg) was subjected to mass spectral analysis (Table II).

(c) ***S. griseus* Cells.** 16-*O*-Acetylvindoline (1a) was also incubated with *S. griseus* cell suspensions. A total of 130 mg of 1a in 10 mL of MeOH was added to 400 mL of *S. griseus* cell suspension, and the mixture was incubated for 24 h. At this stage, approximately 20% of the substrate 1a was converted to a single polar product, and the reaction was terminated by adjustment of the pH to 9.5 with NH_4OH and extraction with ethyl acetate. Preparative layer chromatography was used to remove unreacted 1a from the presumed polar iminium metabolite 2a. The polar metabolite was then dissolved in CH_3OD and reduced with NaBD_4 as described before, and the resulting deuteriated 16-*O*-acetylvindoline (0.6 mg) (1b) was subjected to mass spectral analysis (Table II).

Conversion of an Acetyl Iminium Derivative (2a) to the Enamine Dimer (5). 16-*O*-Acetylvindoline (1a; 10 mg in 0.1 mL MeOH) was added to each of three flasks containing 25 mL of pH 5.0, 0.2 M potassium phosphate solution and 15 units of *P. anceps* laccase. The contents of all the flasks were pooled, adjusted to pH 9.5, and extracted with an equal volume of ethyl acetate. The extracts were dried under vacuum, redissolved in 2 mL of 0.5 N sodium methoxide, and allowed to react on ice with stirring under a stream of nitrogen. The reaction was quenched after 7 h by the addition of 10 mg of Na_2HPO_4 and 5 mL of water. The resulting solution was extracted with chloroform (2 × 10 mL), dried over anhydrous Na_2SO_4 , and evaporated under vacuum to

a pale yellow residue. The residue was further purified over 0.5-mm silica gel GF₂₅₄ developed with solvent system B. The band between R_f 0.60 and 0.85 was scraped from the plate and eluted with acetone to provide a pale yellow residue upon evaporation. The residue contained a mixture of vindoline (1) and the enamine dimer (5) by TLC (solvent systems A and B) and by HPLC when compared with authentic standards.

Trapping of the Iminium Derivative (2a) with Cyanide Ion. 16-*O*-Acetylvindoline (1a; 10 mg in 0.1 mL MeOH) was added to each of five flasks containing 25 mL of pH 5.0, 0.2 M potassium phosphate solution and laccase (23 units), and incubations were conducted as before. The reactions were terminated by adjustment to pH 9.5 with NH_4OH and extraction with 100 mL of ethyl acetate. The extract was evaporated to dryness and redissolved in 3 mL of saturated, methanolic KCN. The cyanide-containing mixture was stirred for 4 h at room temperature before being poured into 3 mL of a saturated solution of NaCl, which was then extracted with chloroform (3 × 10 mL). The organic extracts were pooled and dried to yield 55 mg of crude red oil. TLC of the oil (solvent system C) revealed at least four products plus unreacted starting material. Controls of 16-*O*-acetylvindoline stirred in saturated methanolic KCN showed no product formation by TLC. The oil was purified by passing it through a minicolumn of silica gel (7 g, 1 × 20 cm) using chloroform as eluant, while 3 mL fractions were collected. Fractions 1 and 2 were identical and were combined to yield a product of lower polarity than 16-*O*-acetylvindoline (system C) (2 mg), which was subjected to mass spectral analysis: m/e (relative intensity) 523 (1), 495 (1), 496 (1), 467 (1), 453 (1), 437 (1), 377 (2), 365 (4), 320 (3), 295 (7), 293 (5), 214 (6), 213 (10), 212 (14), 188 (100), 174 (78), 135 (22), 121 (24).

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Registry No. 1, 2182-14-1; 1a, 58811-96-4; 1b, 89691-21-4; 2a, 89709-43-3; 5, 68385-75-1; copper oxidase, 9035-73-8.

Acetylation of Some Novel Hemicholinium Compounds by Soluble Choline Acetyltransferase: Structure-Activity Relationships

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Four bisquaternary nitrogen analogues of 2,2'-[1,1'-biphenyl]-4,4'-diylbis[2-hydroxy-4,4-dimethylmorpholinium] bromide (hemicholinium 3, HC-3) have been synthesized. These analogues differ from HC-3 in that they have a number of methylene groups inserted between the two phenyl rings. This study examines the significance of the internitrogen distance in these compounds with regard to their acetylation by soluble choline acetyltransferase (ChAc) *in vitro*. The hemicholinium compounds were incubated with [¹⁴C]acetylcoenzyme A and any acetylated products were isolated by liquid ion exchange. Only HC-3 and the analogue with three methylene groups between the two phenyl rings, that is, 2,2'-(1,3-propanediyl-di-1,4-phenylene)bis[2-hydroxy-4,4-dimethylmorpholinium] (3CHC), were found to be significantly acetylated. The acetylation rate of both these two compounds was 28% that of choline. It is concluded that an internitrogen distance of 14 Å in bisquaternary nitrogen choline analogues provides the optimum distance for acetylation by ChAc *in vitro*.

Choline is an essential substrate for the synthesis of acetylcholine in cholinergic neurons. This synthesis is catalyzed by the enzyme acetylcoenzyme A:choline *O*-

acetyltransferase (EC 2.3.1.6, choline acetyltransferase, ChAc), the properties of which have recently been reviewed and discussed.¹⁻⁵ A partially purified preparation of this

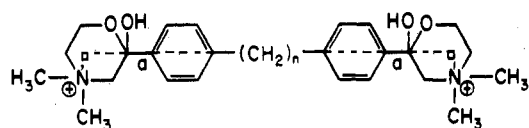
* Roswell Park Memorial Institute.

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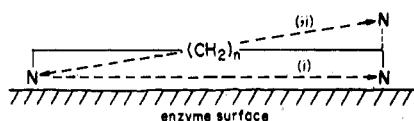
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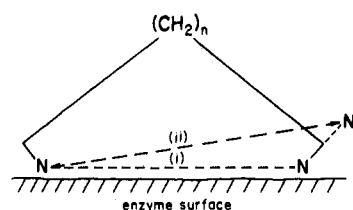
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(A) When $n = 0, 2$ and 4

- (i) minimum internitrogen distance
(ii) maximum internitrogen distance

(B) When $n = 1$ and 3

- (i) minimum internitrogen distance
(ii) intermediate internitrogen distance



(iii) maximum internitrogen distance

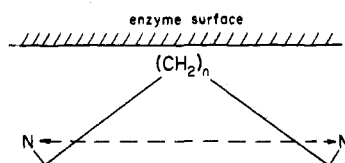


Figure 1. Diagrammatic representation of the molecular structures of HC-3 (1, $n = 0$); 1CHC (2, $n = 1$); 2CHC (3, $n = 2$); 3CHC (4, $n = 3$), and 4CHC (5, $n = 4$), which were used to calculate the internitrogen distances. These distances are altered by rotation about the "a" bonds.

enzyme has been shown to acetylate choline and other choline analogues.⁵⁻¹⁰ One of these analogues is the bis-quaternary nitrogen compound, 2,2'-[1,1'-biphenyl]-4,4'-diylbis[2-hydroxy-4,4-dimethylmorpholinium] (hemicholinium 3, HC-3, 1). This compound was first synthesized by Long and Schueler,¹¹ and its acetylation by ChAc has been studied by several other workers.^{7,12-14}

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Table I. Rates of Acetylation of the Hemicholinium Compounds by ChAc in Vitro

substrate	% acetylation ^a
choline	100 ^b
HC-3 (1)	28
1CHC (2)	4
2CHC (3)	3
3CHC (4)	28
4CHC (5)	5

^a All values were compared to choline. The incubation was carried out for 10 min at 37 °C at a substrate concentration of 20 mM. Experiments were performed in duplicate and repeated five times; \pm SEM were less than 5%.
^b 13.0 ± 1.1 (SEM) μ mol of acetylcholine (g of protein)⁻¹ (10 min)⁻¹.

Table II. Apparent Michaelis-Menten Constants^a for the Acetylation of Choline, HC-3 (1), and 3CHC (4) by ChAc in Vitro

substrate	K_M , mM	V_{max} , μ mol/g of protein
choline	0.29	12.0
HC-3 (1)	1.21	
3CHC (4)	1.27	

^a Calculated from Lineweaver-Burke plots. Incubations were carried out for 10 min at 37 °C in the presence of 18 μ M [¹⁴C]acetylcoenzyme A. Experiments were performed in triplicate and repeated twice; \pm SEM were all less than 10%.

The quaternary nitrogen atom of choline and its analogues is thought to be important in the binding of these molecules to the active site of ChAc. In this study, four analogues (2-5) of HC-3 (1) have been synthesized which have an increasing number of methylene groups between the two phenyl rings (Figure 1). We have, therefore, been able to examine the significance of the interatomic distance between the two quaternary nitrogens in these compounds with regard to their acetylation by soluble ChAc in vitro. Their activity in this respect was compared to choline and HC-3 (1).

Chemistry. The starting materials for the preparation of HC-3 (1) and the analogues 2-5 were diphenyl and the appropriate biphenylalkane compound. The compounds 1-5 were synthesized according to the general method of Long and Schueler¹¹ but with several modifications that were found to improve the yields. The synthetic reaction can be divided into three steps. The first step was a Friedel-Crafts acylation, which resulted in the synthesis of 4,4'-bis(chloroacetyl)-1,1'-biphenyl derivatives 6-10. Excess aluminum chloride was used in this reaction (three times the amount used by Long and Schueler¹¹) and butan-1-ol was used as the recrystallizing solvent. The second step involved a condensation between the 4,4'-bis(chloroacetyl)-1,1'-biphenyl derivatives 6-10 and *N,N*-dimethylethanamine to give the open-chain form of the hemicholinium compounds. Acetone was used as the solvent in this reaction, except in the synthesis of 4CHC (5), where 1,4-dioxane was used. The third step was a cyclization process where the open-chain form of the hemicholinium compounds as solutions in ethanol underwent a spontaneous intramolecular keto-enol (open chain-cyclic) tautomerism to give the cyclic structure of the hemicholinium compounds 1-5.

Biochemical Studies. Table I summarizes the results obtained when the hemicholinium compounds 1-5 were compared to choline as substrates for the soluble choline acetyltransferase enzyme. Only HC-3 (1) and 3CHC (4) were acetylated at a rate of more than 5%, compared to 100% acetylation of choline. This finding for HC-3 (1)

confirms the results of deLores Arnaiz et al.¹² and Bradshaw and Hemsworth,¹⁴ who showed that HC-3 (1) was acetylated by about 20–30%, compared to 100% acetylation of choline. An incubation medium of low ionic strength (40 mM) was used in our experiments, since Hersh et al.⁸ have demonstrated that this will provide optimum conditions for studying the relative rates of acetylation of choline analogues by ChAc. Time studies demonstrated that the rates of acetylation of both HC-3 (1) and 3CHC (4) were linear for at least 10 min at the concentrations employed. At a constant concentration of acetylcoenzyme A (18 μ M), plots of velocity of acetylation as a function of substrate concentration showed typically shaped curves for an enzyme–one substrate reaction. The apparent Michaelis–Menten constants for each substrate were calculated from Lineweaver–Burke¹⁵ plots and are shown in Table II. It was not possible to convert disintegrations per minute to mole of isolated acetylated product for the hemicholinium compounds, since each molecule of HC-3 (1) and 3CHC (4) has two hydroxyl groups, and it is not known whether these compounds are enzymatically acetylated to mono- or biacetylated products.

Our blank values were sufficiently low for us to be able to conclude that the enzyme purification process had removed practically all the endogenous choline. The addition of 0.2 mM *N*-(hydroxymethyl)-4-(1-naphthylvinyl)pyridinium (NVPH), a ChAc inhibitor,¹⁶ inhibited the acetylation of choline, HC-3 (1), and 3CHC (4) by 93–96%, indicating that the acetylation of these compounds is solely due to the enzymatic action of ChAc.

Structure–Activity Relationships. Of the hemicholinium analogues studied, only HC-3 (1) and 3CHC (4) were found to be significantly acetylated *in vitro* by soluble ChAc. Since all the analogues have identical choline moieties, this suggests that the interatomic distance between the two quaternary nitrogens in each molecule is an important factor for determining whether or not the analogues are acetylated. Therefore, this distance has been calculated for each of the compounds, using the bond lengths and angles determined from crystal structures.^{17–19}

The interatomic distance between the two quaternary nitrogens in the HC-3 (1, Figure 1A, $n = 0$) molecule was calculated to be approximately 14 Å. This agrees with the calculations originally made by Long and Schueler.¹¹ Since rotation can occur around the “a” bond, it is possible to obtain a minimum internitrogen distance of 14.1 Å and a maximum of 14.4 Å. The insertion of a methylene group between the two phenyl rings in the HC-3 molecule to give the analogue 1CHC (2, Figure 1B, $n = 1$) reduced the minimum internitrogen distance to 11.0 Å. Again, rotation around the “a” bond can occur, giving a maximum internitrogen distance of 14.4 Å. The insertion of two methylene groups between the two phenyl rings in the HC-3 molecule gave the analogue 2CHC (3). One possible conformation of this molecule is such that the phenyl rings will lie close to each other. However, steric and electronic effects will make this an unstable conformation, which is unlikely to exist. The conformation indicated in Figure 1A ($n = 2$) is more stable, and rotation about the “a” bond

in this conformation gives a minimum internitrogen distance of 16.1 Å and a maximum of 16.7 Å.

The 3CHC (4) molecule can also theoretically exist in a conformation where the phenyl rings will lie close to each other. Again, steric hindrance and electronic repulsion will make this an unstable conformation, which is unlikely to exist. The most stable conformation of this molecule is indicated in Figure 1B ($n = 3$), where rotation about the “a” bond gives a maximum internitrogen distance of 16.9 Å and a minimum of 13.4 Å. The most stable conformation of the 4CHC (5) molecule is shown in Figure 1A ($n = 4$). Rotation about the “a” bond gives a maximum internitrogen distance of 19.0 Å and a minimum of 18.4 Å.

HC-3 (1) and the most stable conformation of 3CHC (4) have a similar interatomic distance between the quaternary nitrogens in their molecular structures. HC-3 (1) possesses a minimum internitrogen distance of 14.1 Å [Figure 1A (i), $n = 0$], and 3CHC (4) possesses a minimum internitrogen distance of 13.4 Å [Figure 1B (i), $n = 3$], a difference of 0.7 Å. Bearing in mind the approximations made in calculating the interatomic distances and the bending of bonds that can occur in solutions, it seems possible for these two structures to occupy the same sites on the enzyme surface. Both quaternary nitrogens in either molecule presumably bind to anionic sites approximately 14 Å apart.

1CHC (2) has a conformation in which the internitrogen distance is similar to that of HC-3, that is, 14.4 Å [Figure 1B, (iii), $n = 1$], but this conformer will not allow both quaternary nitrogen atoms to combine with anionic sites on the same planar surface of the enzyme, since the bulky phenyl groups will sterically hinder the attachment, as shown (Figure 1B, (iii)). Since 1CHC (2) is not acetylated by the enzyme, this suggests that both nitrogen atoms in these hemicholinium compounds not only have to be 14 Å apart in order to be acetylated, but they must also have to bind to the same planar surface of the enzyme.

Hemsworth^{20,21} studied a series of polymethylene dicholine compounds, while Sollenberg et al.²² investigated a series of bisquaternary nitrogen pyridinium choline analogues. Both groups of workers concluded that increasing the internitrogen distance in these analogues improved the compounds as substrates for ChAc *in vitro*. We have calculated the interatomic distances for these molecules and found that the analogues having an internitrogen distance of 8.7 to 13.6 Å had high rates of acetylation, the optimum rate being when the distance was 13.6 Å. The hemicholinium compounds studied here seem to require a more precise internitrogen distance, but, again, about 14 Å is the optimum distance.

These results indicate that the bisquaternary nitrogen choline analogues bind to anionic sites on the ChAc enzyme that are about 14 Å apart. However, if both the quaternary nitrogen atoms bind to active sites, this would mean that these sites are very close to each other on the ChAc enzyme. This is unlikely, since active sites would be expected to be as far away from each other as possible so that the substrates would have easy access to them without any steric interference. Sollenberg et al.²² concluded from their studies that one quaternary nitrogen of the bisquaternary choline analogues binds to the active site, while the other binds to some other anionic group located outside the active site of the enzyme. Such a site could be an acidic amino acid group. Since it is likely that only one nitrogen

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binds to an active site, this suggests that both acetyl-HC-3 and acetyl-3CHC, as synthesized by ChAc, are monoacetylated in vitro.

Experimental Section

Chemical Methods. Melting points were determined in open glass capillaries with an Electrothermal melting point apparatus and are uncorrected. IR spectra were recorded on a Unicam S.P. 200 spectrophotometer. NMR spectra were determined in D₂O on a Varian A60 spectrometer with Me₄Si as an internal standard. All spectral data for the products were consistent with the assigned structures. Microanalysis were performed by the Butterworth Microanalytical Consultancy Ltd., Middlesex, U.K., and all the results were within $\pm 0.4\%$ of the theoretical values.

General Procedures for the Preparation of the 4,4'-Bis-(chloroacetyl)-1,1'-biphenyl Derivatives (6-10). The 1,1'-biphenyl analogue (0.07 mol) was dissolved in a sufficient quantity of carbon disulfide (60 mL). This solution was then cooled, and powdered aluminum chloride (52 g, 0.039 mol) was added slowly with stirring. Chloroacetyl chloride (18.4 g, 0.16 mol) was added dropwise to the mixture, which was then allowed to reflux (no heat required) with stirring, until liberation of hydrogen chloride had ceased (about 3 h). The resulting complex was thoroughly broken up in a mixture of ice, methanol, and hydrochloric acid, and the product was oven dried at 80 °C. The product was recrystallized from butan-1-ol. A cold solution of the product in acetone was passed through activated charcoal until a clear solution was obtained. This solution was then filtered through Kieselguhr, the acetone was removed by vacuum distillation, and the resulting colorless product crystallized from butan-1-ol.

1,1'-Methylenebis[4-(chloroacetyl)benzene] (7). 1,1'-Methylenebis[benzene] (11.8 g, 0.07 mol) gave brown needles (18.7 g, 83%), which were decolorized to give colorless crystals of product, mp 115-117 °C. Anal. (C₁₇H₁₄O₂Cl₂) C, H, O.

1,1'-(1,2-Ethanedyl)bis[4-(chloroacetyl)benzene] (8). 1,1'-(1,2-Ethanedyl)bis[benzene] (12.7 g, 0.07 mol) gave yellow needles (19.2 g, 82%), which were decolorized to give colorless crystals of product, mp 123 °C. Anal. (C₁₈H₁₆O₂Cl₂) C, H, O.

1,1'-(1,3-Propanedyl)bis[4-(chloroacetyl)benzene] (9). 1,1'-(1,3-Propanedyl)bis[benzene] (13.7 g, 0.07 mol) gave yellow needles (13.9 g, 57%) which were decolorized to give colorless crystals of product: mp 131-133 °C. Anal. (C₁₉H₁₈O₂Cl₂) C, H, O.

1,1'-(1,4-Butanedyl)bis[4-(chloroacetyl)benzene] (10). 1,1'-(1,4-Butanedyl)bis[benzene] (14.7 g, 0.07 mol) gave yellow needles (13.72 g, 54%), which were decolorized to give colorless crystals of product: mp 149-151 °C. Anal. (C₂₀H₂₀O₂Cl₂) C, H, O.

General Procedures for the Preparation of the 2,2'-[1,1'-Biphenyl]-4,4'-diylbis[2-hydroxy-4,4-dimethylmorpholinium] Dichloride Analogues (1-5). The appropriate 4,4'-bis(chloroacetyl)-1,1'-biphenyl analogue (6-10; 0.03 mol) was dissolved in boiling acetone, and 2-(dimethylamino)ethanol (7.4 g, 0.08 mol) was added. The mixture was then allowed to cool without stirring for 5 h. The precipitated product was crystallized from ethanol/ether. The ethanol/ether mixture containing the crystals was centrifuged and the precipitate was washed twice with fresh ether, which was finally evaporated under vacuum. The product was dried in a vacuum desiccator. The analogues were decolorized by dissolving in ethanol, and, before precipitation with ether, the cold solution was passed through charcoal and finally through Kieselguhr.

2,2'-[1,1'-Biphenyl]-4,4'-diylbis[2-hydroxy-4,4-dimethylmorpholinium] Dichloride (Hemicholinium 3 as a Dichloride Salt, HC-3) (1). 4,4'-Bis(chloroacetyl)-1,1'-biphenyl (6; 9.2 g, 0.03 mol) gave colorless microneedles of product (10.3 g, 71%): mp 222 °C; IR (Nujol), 3400-3200 (associated hydroxy), 1600 (bi-

phenyl), 1080 (morpholinium ether), 1000 (aromatic), 820 (*p*-phenyl). Anal. (C₂₄H₃₄N₂O₄Cl₂) C, H, N, O.

2,2'-(Methylenedi-1,4-phenylene)bis[2-hydroxy-4,4-dimethylmorpholinium] Dichloride (1CHC; 2). 1,1'-Methylenebis[4-(chloroacetyl)benzene] (7; 9.63 g, 0.03 mol) gave brown crystals (7.8 g, 52%), which were decolorized to give colorless microneedles of product, mp 199-202 °C. Anal. (C₂₅H₃₆N₂O₄Cl₂) C, H, N, O.

2,2'-(1,2-Ethanedyl)di-1,4-phenylene)bis[2-hydroxy-4,4-dimethylmorpholinium] Dichloride (2CHC; 3). 1,1'-(1,2-Ethanedyl)bis[4-(chloroacetyl)benzene] (8; 10.1 g, 0.03 mol) gave yellow crystals (14.5 g, 94%), which were decolorized to give colorless microneedles of product, mp 189 °C. Anal. (C₂₆H₃₈N₂O₄Cl₂) C, H, N, O.

2,2'-(1,3-Propanedyl)di-1,4-phenylene)-4,4'-diylbis[2-hydroxy-4,4-dimethylmorpholinium] Dichloride (3CHC; 4). 1,1'-(1,3-Propanedyl)bis[4-(chloroacetyl)benzene] (9; 10.5 g, 0.03 mol) gave yellow crystals (10.3 g, 65%), which were decolorized to give colorless microneedles of product, mp 176-178 °C. Anal. (C₂₇H₄₀N₂O₄Cl₂) C, H, N, O.

2,2'-(1,4-Butanedyl)di-1,4-phenylene)-4,4'-diylbis[2-hydroxy-4,4-dimethylmorpholinium] Dichloride (4CHC; 5). 1,1'-(1,4-Butanedyl)bis[4-(chloroacetyl)benzene] (10; 10.9 g, 0.03 mol), with 1,4-dioxane as the solvent, gave yellow crystals (11.2 g, 69%), which were decolorized to give colorless microneedles of product, mp 196-198 °C. Anal. (C₂₈H₄₂N₂O₄Cl₂) C, H, N, O.

Biochemical Methods. A partially purified extract of soluble ChAc was prepared from rat brain. Acetone dried powders were first prepared by the method described by Hemsworth and Morris.²³ When dry, the powders were extracted following the method of Mann and Hebb.²⁴ The rates of acetylation of choline and the hemicholinium compounds were estimated by incubating these compounds with [¹⁴C]acetylcoenzyme A and ChAc, followed by a quantitative determination of the amount of radioactively labeled acetylated product formed.

The incubation mixture was placed in a plastic microfuge tube (Beckman) and contained 5 μ L of ChAc, 10 μ L of choline or hemicholinium compound at the required concentration, and 10 μ L of a buffer containing [¹⁴C]acetylcoenzyme A and other constituents [final concentration, in mM: NaCl, 12.0; MgSO₄, 2.0; disodium EDTA, 0.04; potassium phosphate (pH 7.7), 6.0; phytostigmine sulfate, 0.08; [¹⁴C]acetylcoenzyme A, 0.018 (59.5 mCi/mmol, The Radiochemical Centre, Amersham, England); bovine serum albumin, 0.02%, w/v]. Blank values were determined by incubating the enzyme and buffer with distilled water. All additions were made at 4 °C, and, when complete, the tubes were shaken and then transferred to a shaking water bath and incubated at 37 °C for 10 min. The radioactive [¹⁴C]acetylated product was extracted into a toluene-based scintillation fluor by liquid ion exchange, as described by Fonnum.²⁵

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Registry No. 1, 18997-39-2; 2, 89302-44-3; 3, 89302-45-4; 4, 89302-46-5; 5, 89302-47-6; 6, 24860-53-5; 7, 37494-06-7; 8, 68114-93-2; 9, 67442-67-5; 10, 67442-69-7; Ph₂CH₂, 101-81-5; Ph(CH₂)₂Ph, 612-00-0; Ph(CH₂)₃Ph, 1530-03-6; Ph(CH₂)₄Ph, 1083-56-3; CH₂ClC(O)Cl, 79-04-9; N(CH₃)₂CH₂CH₂OH, 108-01-0; EC 2.3.1.6, 9012-78-6.

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