O-Carbamoylsalicylates: Agents for Modification of Hemoglobins

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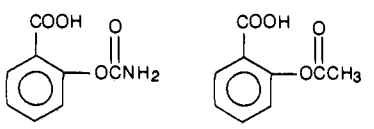
To combine the attractive features of cyanate and of O-acetylsalicylate as hemoglobin-modifying agents we have prepared carbamoylsalicylate. This compound is a close analogue of aspirin and also resembles a masked cyanate. O-Carbamoylsalicylate and some related carbamates modify hemoglobin substantially, even at 5 mM concentration.

Various strategies have been considered for modifying hemoglobin S to decrease its sickling tendencies. Chemical approaches can be based on covalent or noncovalent interactions. From a molecular viewpoint, if covalent approaches are to be used, the most attractive sites for modification with reagents mild enough to be pharmacologically acceptable are the amine-containing residues in the protein. Among amine-blocking reagents are alkylating compounds, aldehydes that form Schiff bases, amidinating reagents, carbamylating compounds, and acylating agents.

Sodium cyanate has been shown[1,2] to carbamoylate hemoglobin extensively, largely at the N-terminal amines of the α and β chains, and to be an inhibitor of sickling. Subsequently, however, evidence of toxicity appeared in clinical studies[3,4] with cyanate. Among possible acylating agents, aspirin derivatives have been particularly attractive[5,6] since they are mild and have a long pharmacological history of acceptance. It occurred to us that it might be possible to combine features of both types of reagent by preparing O-carbamoylsalicylate (I). Carbamates have a long record of pharmacological acceptability. This molecule (I) is a close analogue of O-acetylsalicylate (II) and at the same time resembles a masked cyanate. Thus one might expect it to be a good carbamoylating agent without presenting the risks of circulating free cyanate in in vivo use.



I II

Chemistry. Four different procedures, based on literature descriptions for the synthesis of other O-carbamates, were investigated. The first,[7] using the pertinent phenol and 2 molar equiv of sodium cyanate and of trifluoroacetic acid in nonpolar solvents, failed to yield the carbamate of either salicylic acid or its methyl ester. Among the solvents tried were dichloromethane, anhydrous ether, tetrahydrofuran, and 1,2-dimethoxyethane, and temperatures were varied from 25 °C to 50 °C. In a second procedure with carbon tetrachloride as solvent and trichloroacetic acid[8] instead of trifluoroacetic, at 55 °C, only starting reactants were recovered. Alternative solvents such as chloroform, benzene, or dimethoxyethane were no more effective, even though the reaction worked well with simple, unsubstituted phenol.

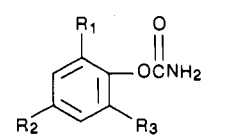
Table I. Analyses of the Carbamates

compd	empirical formula	elemental analysis, %: calcd (found) C	H	N
IV	$C_9H_9NO_4$	55.38 (55.41)	4.65 (4.66)	7.18 (7.14)
V	$C_9H_9NO_4$	55.38 (55.23)	4.65 (4.59)	7.18 (7.09)
VI	$C_{15}H_{13}NO_4$	66.41 (66.17)	4.83 (4.91)	5.16 (5.18)
I	$C_8H_7NO_4$	53.04 (52.79)	3.90 (4.12)	7.73 (7.61)
VII	$C_{15}H_{11}Br_2NO_4$	41.98 (41.64)	2.58 (2.77)	3.27 (3.32)
VIII	$C_{17}H_{17}NO_4$	68.21 (67.72)	5.73 (5.76)	4.68 (4.59)
IX	$C_{10}H_{11}NO_4 \cdot 0.5C_2H_5OH \cdot H_2O$	52.80 (53.28)	6.39 (5.67)	5.60 (6.17)
X	$C_{17}H_{15}Br_2NO_4$	44.66 (44.26)	3.31 (3.33)	3.06 (3.07)

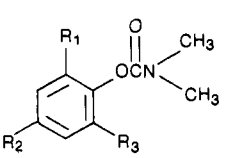
In analogy with the biochemical carbamoylating agent carbamoyl phosphate ($H_2NC(O)OPO_3H_2$, which converts ornithine to citrulline), we devised a third procedure using carbamoyl azide, $H_2NC(O)N_3$, as active reagent. Attempts to carbamoylate methyl salicylate at room temperature in dichloromethane, chloroform, or a 6:4 mixture of chloroform and dimethoxyethane, with equimolar quantities of carbamoyl azide, in the presence of triethylamine or N,N-dimethylaniline as base, led only to starting salicylate ester. With triethylamine, cyanuric acid was also generated. Its identity was confirmed by its melting point (>350 °C) and infrared spectrum[9] (in KBr).

Although azidoformate esters such as $(CH_3)_3COC(O)N_3$ and $CH_3OC_6H_4CH_2OC(O)N_3$ react with amines to displace azide anion and form the respective carbamates, no reaction was obtained with $H_2NC(O)N_3$. The lack of reactivity of carbamoyl azide probably reflects the reduced susceptibility to nucleophilic displacement at the carbonyl carbon due to resonance stabilization in the structure, $H_2NC(O)N_3 \leftrightarrow H_2N^+=C(O^-)N_3$. The formation of cyanuric acid from carbamoyl azide is probably the consequence of proton abstraction by triethylamine followed by displacement of the azide ion to give HN=C=O, which in turn proceeds to cyanuric acid.

The desired carbamates were finally prepared in a reaction of the carboxyl-blocked phenol with chlorosulfonyl isocyanate[10] followed by hydrolysis of the intermediate adduct $ArOC(O)NHSO_2Cl$. By this procedure a number of benzyl and methyl esters, IV–VII, were synthesized. To reach O-carbamoylsalicylic acid (I), the benzyl group was removed from the corresponding carboxylic ester (VI) by catalytic hydrogenolysis.



III: $R_1=R_2=R=H$
IV: $R_2=COOCH_3$, $R_1=R_3=H$
V: $R_1=COOCH_3$, $R_2=R_3=H$
VI: $R_1=COOCH_2C_6H_5$, $R_2=R_3=H$
I: $R_1=COOH$, $R_2=R_3=H$
VII: $R_1=COOCH_2C_6H_5$, $R_2=R_3=Br$



VIII: $R_1=COOCH_2C_6H_5$, $R_2=R_3=H$
IX: $R_1=COOH$, $R_2=R_3=H$
X: $R_1=COOCH_2C_6H_5$, $R_2=R_3=Br$

(1) Cerami, A.; Manning, J. M. *Proc. Natl. Acad. Sci. U.S.A.* **1971**, *68*, 1180.
(2) Njikam, N.; Jones, W. M.; Nigen, A. M.; Gillette, P. N.; Williams, R. C., Jr.; Manning, J. M. *J. Biol. Chem.* **1973**, *248*, 8052.
(3) Harkness, D. R.; Roth, S. *Prog. Hematol.* **1975**, *9*, 157.
(4) Peterson, C. M.; Tsairis, P.; Ohnishi, A.; Lu, Y. S.; Grady, R.; Cerami, A.; Dyck, P. J. *Ann. Intern. Med.* **1974**, *81*, 152.
(5) Klotz, I. M.; Tam, J. W. O. *Proc. Natl. Acad. Sci. U.S.A.* **1973**, *70*, 1313.
(6) Klotz, I. M.; Haney, D. N.; King, L. C. *Science (Washington, D.C.)* **1981**, *213*, 724.
(7) Loev, B.; Kormendy, M. F. *J. Org. Chem.* **1963**, *28*, 3421.
(8) Al-Rawi, H.; Williams, A. *J. Am. Chem. Soc.* **1977**, *99*, 2671.
(9) Newman, R.; Badger, R. M. *J. Am. Chem. Soc.* **1952**, *74*, 3545.
(10) Graf, R. *Angew. Chem., Int. Ed. Engl.* **1968**, *7*, 172.

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Table II. Modification of Hemoglobin by Carbamoyl Derivatives of Salicylates[a]

compd	concn, mM	% modification of hemoglobin[b] oxy	% modification of hemoglobin[b] deoxy	oxygen affinity: p_{50}, mmHg no IHP[c]	oxygen affinity: p_{50}, mmHg with 2 mM IHP
none (control)	0	0	0	6	46
III	10	67	nd	5	30
	5	44	40	6	39
IV	10	89	nd	6.5	23
	5	61	58	6	33
V	10	83	nd	6	25
	5	54	52	6	35
VI	5[d]	45	46	6	37.5
I	10	94	nd	6	22
	5	62	58	6	33
VIII	10	0	nd	6	44
	5	0	0	6	42
IX	10[d]	0	nd	6	42
X	10	0	nd	7	41
	5	0	0	6	41

[a] Modification experiments were carried out in 0.1 M Tris·HCl buffer, pH 7.3, 37 °C for 2 h. The extent of modification and oxygen affinities were determined as described previously (ref 17). [b] Hemoglobin A was used at 1.0 mM concentration. [c] IHP, inositol hexaphosphate. [d] Concentration of the compounds is uncertain due to incomplete solubility. The notation "nd" indicates "not determined".

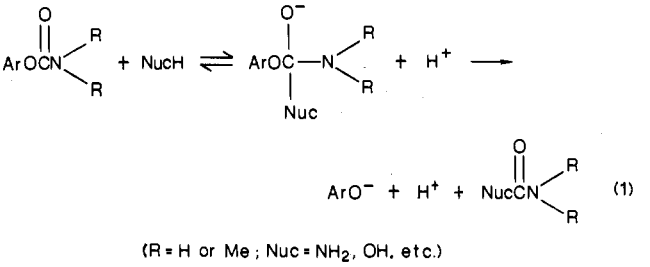
The *N,N*-dimethyl derivatives VIII–X were synthesized in a reaction of *N,N*-dimethylcarbamoyl chloride with the appropriate benzyl ester. The solvent was anhydrous benzene or dimethylformamide, the base *N*-methylmorpholine, and the temperature 60 °C. Again to obtain *O*-(dimethylcarbamoyl)salicylic acid (IX), the benzyl group was removed from VIII by hydrogenolysis.

Analytical data from the compounds prepared are assembled in Table I.

Modification of Hemoglobin. The effects of the *O*-carbamoyl- and *O*-(dimethylcarbamoyl)salicylate derivatives on modification of hemoglobin and on its oxygen affinity are summarized in Table II.

From this table it is evident that whereas all of the N-unsubstituted carbamates were effective in modifying hemoglobin, even at 5 mM concentration, none of the *N,N*-dimethylcarbamates, VIII–X, showed any effect. There were no significant differences between the oxy and deoxy forms of hemoglobin in the modification by carbamates I and III–VI.

The striking difference in effectiveness of the N-unsubstituted vs. the *N,N*-dimethyl-substituted carbamates probably reflects the mechanism by which these two sets of compounds react with the nucleophiles (such as the ϵ- or α-amino groups) of hemoglobin. Previous investigations[8,11-13] of simple and substituted phenol carbamates suggest two possible pathways by which *O*-carbamates can react with nucleophiles: (i) by direct nucleophilic displacement at the carbamoyl carbon followed by aroxide anion release, and (ii) by the so-called elimination–addition mechanism[8,11] wherein proton abstraction from carbamate NH_2 by a base followed by release of aroxide anion gives the reactive isocyanic acid, HN=C=O (or isocyanate if the N is monosubstituted), which subsequently reacts with the nucleophile (for example, an amino group to give the N-carbamoylated product). These reactions are illustrated in eq 1 and 2. Although only pathway (1) is accessible



(R = H or Me; Nuc = NH_2, OH, etc.)

$$ArOC(O)NH_2 \overset{base}{\rightleftharpoons} ArO^- + H^+ + HN{=}C{=}O \quad (2)$$

$$HN{=}C{=}O + NucH \rightarrow NucC(O)NH_2$$

to the *N,N*-dimethylcarbamoyl derivatives, both pathways are feasible for the carbamates with either one or none of the hydrogens on the NR_2 substituted. Since none of the *N,N*-dimethylcarbamates, VIII–X, produced any modification of hemoglobin, whereas substantial modification was found with the *O*-carbamoyl derivatives, I and III–VII, it seems likely that pathway (2) is the mechanism of modification of hemoglobin by the N-unsubstituted carbamates.

Effects on Oxygen Affinity. All of the treated hemoglobins, whether carbamoylated or not, bound oxygen in the absence of inositol hexaphosphate, with a p_{50} value of about 6 mm, the same as that shown by untreated hemoglobin. Similarly, the samples of protein exposed to the *N,N*-dimethylcarbamates showed a p_{50} in the presence of inositol hexaphosphate near that (46 mm) of untreated hemoglobin. This parallelism is consistent with the absence of any indication of modification by the *N,N*-dimethylcarbamates. However, with the N-unsubstituted carbamates, all of which modified hemoglobin, the effect on p_{50} in the presence of inositol hexaphosphate depended on the concentration of reagent used, which determined the extent of modification. In general, at 5 mM concentration of carbamate, the p_{50} was lowered to a value near 35 mm. On the other hand at the higher concentration of reagent, p_{50} was lowered to 22–30 mm. The drop below 46 mm, that of untreated protein, is a measure of the extent of reaction in the β-cleft region of hemoglobin, for insertion of groups in this domain blocks the binding of inositol hexaphosphate therein[14-17] and hence vitiates the usual response to this effector. We conclude, therefore, that at higher concentration the carbamoylating agents react more extensively with nucleophilic groups in the β-cleft region of hemoglobin. In view of their relatively lower pK_a values, the terminal α-NH_2 groups of the α and β subunits are the most likely sites of carbamoylation.

The extensive modification of hemoglobin by these *O*-carbamoylsalicylates, even at 5 mM concentration, suggests that they may be effective antisickling agents.

Experimental Section

General Procedures. Elemental analyses were performed by Micro-Tech Laboratories, Skokie, IL. Melting points were determined with a Büchi apparatus and are uncorrected. Infrared spectra were recorded with a Perkin-Elmer Model 283 spectrophotometer. Proton NMR spectra were recorded on a 90-MHz Varian-EM 390 instrument with tetramethylsilane (Me_4Si) as the

(11) Dittert, L. W.; Higuchi, T. *J. Pharm. Sci.* **1963**, *52*, 852.
(12) Hegarty, A. F.; Frost, L. N.; Coy, J. H. *J. Org. Chem.* **1974**, *39*, 1089.
(13) Hegarty, A. F.; Frost, L. N. *J. Chem. Soc., Perkin Trans. 2* **1973**, 1719.
(14) Walder, J. A.; Zaugg, R. H.; Walder, R. Y.; Steele, J. M.; Klotz, I. M. *Biochemistry* **1979**, *18*, 4265.
(15) Wood, L. E.; Haney, D. N.; Patel, J. R.; Clare, S. E.; Shi, G.-Y.; King, L. C.; Klotz, I. M. *J. Biol. Chem.* **1981**, *256*, 7046.
(16) Delaney, E. J.; Massil, S. E.; Shi, G.-Y.; Klotz, I. M. *Arch. Biochem. Biophys.* **1984**, *228*, 627.
(17) Massil, S. E.; Shi, G.-Y.; Klotz, I. M. *J. Pharm. Sci.* **1984**, *73*, 1851.

internal standard, and chemical shifts were expressed in parts per million (δ). Thin-layer chromatography was performed with Whatman K6F silica gel glass plates, and ultraviolet, iodine, or ferric chloride (for phenolics) was used as the visualizing agent. The following solvent compositions (v/v) were employed for thin-layer chromatography: (A) chloroform–methanol (10/2), (B) ethyl acetate–petroleum ether (2/3). The reaction solvents, except tetrahydrofuran, were dried over 4A molecular sieves; tetrahydrofuran was dried over neutral alumina. Anhydrous sodium sulfate was used to dry organic extracts following workup of the reaction mixtures.

Synthetic Procedures. Method A. Essentially the procedure of Loev and Kormendy[7] was followed. Experiments were performed with salicylic acid as well as with methyl salicylate as the phenol substrate. Regardless of the reaction solvent (dichloromethane, tetrahydrofuran, 1,2-dimethoxyethane, or anhydrous ether) and reaction conditions (18 h at room temperature or 18–24 h at 45–50 °C), no carbamate product was obtained, only starting material (in 80–90% yield), as confirmed by NMR, thin-layer chromatographs, and melting and mixture melting point.

Method B. The preparation of the known phenol carbamate (III) is an example of this procedure.[8] To a stirred solution of phenol (0.94 g, 10.0 mmol) in 10 mL of CCl_4 was added powdered sodium cyanate (1.3 g, 20 mmol). This was followed by a solution of trichloroacetic acid (3.3 g, 20 mmol) in 10 mL of CCl_4. The reaction mixture was stirred and heated at 55 °C for 3 h (under reflux-condenser cooling and drying tube protection) and then allowed to cool to room temperature. The mixture was stirred with 25 mL of water to dissolve the solids and extracted with CCl_4 (2 × 40 mL). The aqueous layer along with some suspended solid was reextracted with chloroform (2 × 45 mL). The organic extracts were separately washed with water and saturated sodium chloride and dried. Concentration of the CCl_4 extract followed by addition of petroleum ether gave a low-melting (mp 105–109 °C) material in low yield (0.12 g) containing a phenolic group ($FeCl_3$ test) and was not pursued further. The desired phenol carbamate (III) was isolated from the chloroform extract (yield 0.82 g, 60%): mp 147–149 °C ($CHCl_3$–petroleum ether) (lit.[7] mp 145–148 °C); ^1H NMR ($CDCl_3$–Me_2SO-d_6, 2:1) δ 7.0–7.6 (5 H, m, aromatics), 6.38 (2 H, s, br, OCONH_2); TLC R_f 0.76 solvent in A, 0.1 in solvent B. This method, however, failed to give the carbamates from salicylic acid or methyl salicylate regardless of the solvent used (CCl_4, chloroform, benzene, or 1,2-dimethoxyethane). As in the synthetic method A above, these experiments led to the recovery of starting materials in almost quantitative yield.

Method C. Carbamoyl Azide as the Carbamoylating Agent. Carbamoyl azide was prepared essentially according to the literature[8] procedure in 75% yield: mp 95–97 °C (ether–petroleum ether) (lit.[8] mp 96–97 °C); IR (KBr) cm^{-1} 2170 (azide), 3390, 3250 (NH), 1680 (amide I C=O). Reactions were carried out at room temperature for 17–24 h with either methyl salicylate or methyl 4-hydroxybenzoate as the phenolic substrate, in dichloromethane solvent, with equimolar quantities of carbamoyl azide and either triethylamine or *N,N*-dimethylaniline. These procedures gave only starting material in about 95% yield (isolated). The NMR spectra showed the presence of phenolic OH at δ 10.75 and 9.9 for the respective starting materials and revealed no carbamate (OCONH_2) signal at about δ 6.7–6.8. With triethylamine the reaction gave also cyanuric acid in about 95% yield, presumably formed by trimerization of isocyanic acid derived from carbamoyl azide. This identity was established by the melting point (>350 °C) and IR (KBr), which were identical with those of an authentic specimen.[9] With 4-(dimethylamino)pyridine (0.3–1.0 molar equiv) as the base in the above reactions, the carbamate product was formed in about 10% yield as determined from the NMR signals of the phenolic OH at δ 9.9–10 and the carbamate (OCONH_2) at δ 6.7. Thus these reactions too gave predominantly starting materials.

Method D.[10] Reaction of the Phenol with Chlorosulfonyl Isocyanate. Methyl 4-(Carbamoyloxy)benzoate (IV). To a stirred slurry of methyl 4-hydroxybenzoate (0.304 g, 2.0 mmol) in 2.0 mL of dry chloroform was added 0.18 mL (2.0 mmol) of chlorosulfonyl isocyanate under nitrogen. The reaction mixture was stirred at room temperature for 3 h and the solvent removed in vacuo. The residue was stirred with about 5.0 mL of ice-cold water containing crushed ice. The solution was extracted with ethyl acetate (50 mL), and the ethyl acetate extract was washed with water (2 × 10 mL) followed by saturated sodium chloride (3 × 10 mL) and then dried. The organic extract was concentrated to a 10-mL volume and diluted with petroleum ether (40 mL). The crystalline product was filtered off, washed with petroleum ether, and recrystallized from ethyl acetate–petroleum ether to give 0.12 g (31%) of product: mp 146–148 °C; ^1H NMR ($CDCl_3$–Me_2SO-d_6, 3:1) δ 7.15–7.30 (2 H, d, aromatics), 7.9–8.2 (2 H, d, aromatics), 6.77 (2 H, br, s, $OCONH_2$), 3.88 (3 H, s, OCH_3); IR (KBr) cm^{-1} 3440 (NH), 1680 (aryl ester), 1730, 1710 (carbamate), 1215 (COC of ester); TLC R_f 0.89 in solvent A. The elemental analysis is listed in Table I.

An improved yield of 72% of the above carbamate, with a higher melting point (160–162 °C) and a ^1H NMR spectrum identical with that of the lower melting form described above, was obtained when the reaction was carried out with 30% molar excess of chlorosulfonyl isocyanate in dichloromethane for 1.0 h followed by a similar workup.

Methyl *O*-Carbamoylsalicylate (V). When methyl salicylate in chloroform was used in the reaction of method D, it was converted to compound V: yield 0.12 g (12%); mp 135–137 °C (ethyl acetate–petroleum ether); ^1H NMR ($CDCl_3$–Me_2SO-d_6, 3:1) δ 7.0–8.0 (4 H, m, aromatics), 6.65 (2 H, br, s, $OCONH_2$), 3.83 (3 H, s, OCH_3); IR (KBr) cm^{-1} 3305, 3410 (NH), 1725, 1705 (carbamate and aryl ester, respectively), 1250 (COC of ester). Elemental analysis of the product is listed in Table I.

A higher melting form (mp 141–143 °C) of compound V was obtained in a 25% yield when the reaction was carried out with a 30% molar excess of chlorosulfonyl isocyanate in dry dichloromethane for 1.0 h. The NMR spectrum of this product was identical with that of the analytical sample listed in Table I.

Benzyl *O*-Carbamoylsalicylate (VI). Chlorosulfonyl isocyanate (0.95 mL, 10.5 mmol) was added during 5 min to a stirred solution of benzyl salicylate (1.82 g, 8.0 mmol) in 5 mL of dry chloromethane under nitrogen. The reaction mixture was stirred at room temperature for 2.0 h and subjected to a similar workup as for compounds IV and V to give VI as white crystals: yield 1.84 g (85%); mp 115–116 °C (ethyl acetate–petroleum ether); ^1H NMR ($CDCl_3$) δ 7.1–8.2 (9 H, m, aromatics), 5.33 (2 H, s, $OCH_2C_6H_5$), 5.1 (2 H, br, s, $OCONH_2$); TLC R_f 0.89 in solvent A. Elemental analysis is given in Table I.

***O*-Carbamoylsalicylic Acid (I).** To a solution of benzyl *O*-carbamoylsalicylate (0.813 g, 3.0 mmol) in 50 mL of absolute ethanol was added 100 mg of 5% Pd on activated carbon catalyst, and the mixture was hydrogenated at 10 psi for 3.0 h. After removal of the catalyst by filtration, the filtrate was concentrated to a 5.0-mL volume and cooled overnight at 0 °C. The crystalline product was filtered off, washed with absolute ethanol followed by anhydrous ether, and then dried (over P_2O_5 under vacuo) to give 0.268 g (49%): mp 128–130 °C (absolute ethanol–anhydrous ether); ^1H NMR (Me_2SO-d_6) δ 12.8 (1 H, br, COOH), 6.7–8.0 (6 H, m, phenyl + s, due to $OCONH_2$ centered at 6.95); TLC R_f 0.19 in in solvent A. Elemental analysis is given in Table I.

Benzyl *O*-Carbamoyl-3,5-dibromosalicylate (VII). The starting benzyl 3,5-dibromosalicylate was prepared as follows. A mixture of dry, powdered potassium 3,5-dibromosalicylate (3.04 g, 9.0 mmol), tetrabutylammonium bromide (0.29 g), and benzyl bromide (1.4 mL, 12.0 mmol) in 10.0 mL of dry dimethylformamide was stirred at 90–95 °C for 70 h. Dimethylformamide was removed in vacuo and the residue was distributed between ethyl acetate (150 mL) and water (30 mL). The ethyl acetate extract was washed successively with 0.5 N HCl, 4.0% sodium carbonate, water, and saturated sodium chloride and then dried. Concentration of the ethyl acetate extract followed by addition of petroleum ether gave crystalline benzyl 3,5-dibromosalicylate: yield (2.24 g, 64%); mp 109–110 °C (ethyl acetate–petroleum ether); ^1H NMR ($CDCl_3$) δ 11.45 (1 H, s, phenolic OH), 7.93, 8.05 (2 H, dd, substituted aryl), 7.47 (5 H, s, phenyl of benzyl), 5.45 (2 H, s, $OCH_2C_6H_5$); TLC R_f 0.9 in solvent A, 0.8 in solvent B. Anal. Calcd for $C_{14}H_{10}Br_2O_3$: C, 43.55; H, 2.61. Found: C, 43.88; H, 2.54. The same compound was also obtained in 30% yield by the acid-catalyzed esterification of 3,5-dibromosalicylic acid with *p*-toluenesulfonic acid and excess of benzyl alcohol in refluxing benzene for 72 h in a Dean–Stark apparatus. The benzyl 3,5-dibromosalicylate was mixed with a 50% molar excess of chlorosulfonyl isocyanate in dry dichloromethane and allowed to stand

at room temperature for 6.0 h. (Use of less reagent or shorter time resulted in the recovery of starting material with less than 2% of product.) A workup similar to that used for compounds IV–VI gave benzyl *O*-carbamoyl-3,5-dibromosalicylate (VII) as white crystals: yield (0.12 g, 12.7%); mp 170–172 °C (ethyl acetate–petroleum ether); TLC R_f 0.8 in solvent A; product was UV positive but gave negative $FeCl_3$ test, whereas starting compound with R_f 0.94 was UV and $FeCl_3$ positive); ^1H NMR ($CDCl_3$–Me_2SO-d_6, 5:1) δ 7.85, 7.98 (2 H, dd, substituted aryl), 7.4 (5 H, s, phenyl of benzyl), 6.9 (2 H, br, s, $OCONH_2$), 5.3 (2 H, s, $OCH_2C_6H_5$). Elemental analysis is given in Table I.

The mother liquor from the above crystallization gave 65% of starting material, benzyl 3,5-dibromosalicylate, as confirmed by NMR and melting point and mixture melting point.

Benzyl *O*-(Dimethylcarbamoyl)salicylate (VIII). To a stirred solution of benzyl salicylate (1.82 g, 8.0 mmol) in 8.0 mL of dry dimethylformamide was added *N*-methylmorpholine (1.12 mL, 10.0 mmol) followed by 4-(dimethylamino)pyridine (0.25 g, 2.0 mmol) and dimethylcarbamoyl chloride (0.92 mL, 10.0 mmol). The reaction mixture was stirred at 60 °C for 22 h and dimethylformamide was removed under reduced pressure. The residue was distributed between cold dilute (0.5 N) HCl and ethyl acetate. The ethyl acetate extract was washed with water and saturated sodium chloride and then dried. The concentrated extract was diluted with petroleum ether and cooled, and the precipitated product was recrystallized from petroleum ether to which a few drops of ethyl acetate had been added. After several hours at −10 °C, the crystalline product was filtered off and dried to give 1.19 g (50%); mp 45–47 °C; TLC R_f 0.4 in solvent B; UV positive, $FeCl_3$ negative, whereas starting benzyl salicylate, R_f 0.8, was UV and $FeCl_3$ positive; ^1H NMR ($CDCl_3$) δ 7.0–8.0 (9 H, m, aryl + s, phenyl of benzyl with the latter centered at 7.33), 5.25 (2 H, s, $OCH_2C_6H_5$), 2.83 (6 H, s, $N(CH_3)_2$). Elemental analysis is listed in Table I.

***O*-(Dimethylcarbamoyl)salicylic Acid (IX).** The benzyl ester precursor VIII (1.0 g, 3.34 mmol) was catalytically hydrogenated, by the procedure described for the synthesis of I and VI, to give the deblocked product IX as an oil, which failed to crystallize from absolute ethanol–ether. Recrystallization from anhydrous ether–petroleum ether gave a low-melting solid, an oil at room temperature: yield 87%; TLC R_f 0.5 in solvent A, 0.1 in solvent B; ^1H NMR ($CDCl_3$) δ 9.75 (1 H, br, COOH), 7.1–8.15 (4 H, m, aromatics), 3.05 (6 H, d with 7-Hz separation, $N(CH_3)_2$). The NMR spectrum also showed the presence of ethanol with a (3.5 q and 1.2 t) which was not removable despite several hours of drying in vacuo. Elemental analysis is listed in Table I.

Benzyl *O*-(Dimethylcarbamoyl)-3,5-dibromosalicylate (X). Benzyl 3,5-dibromosalicylate was mixed with 50% molar excess of the reagent dimethylcarbamoyl chloride in refluxing pyridine or in benzene with 150% molar excess of the reagent and *N*-methylmorpholine. Refluxing was continued for 18 h. After a similar workup to the reaction mixture as that for compound VIII, *O*-dimethylcarbamoyl derivative X was obtained as white crystals: yield 41%; mp 89–90 °C (ethyl acetate–petroleum ether); TLC R_f 0.6 in solvent B (starting material R_f 0.8); ^1H NMR ($CDCl_3$) δ 8.16, 7.96 (2 H, dd, substituted aryl), 7.45 (5 H, s, phenyl of benzyl), 5.33 (2 H, s, $OCH_2C_6H_5$), 2.9 (6 H, d with 6-Hz separation, $N(CH_3)_2$). Elemental analysis is listed in Table I.

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Imidazole Anticonvulsants: Structure–Activity Relationships of [(Biphenylyloxy)alkyl]imidazoles

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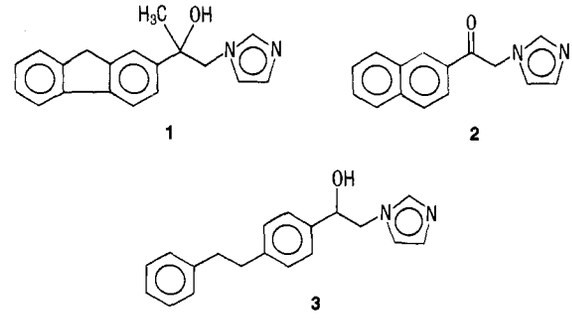
The [(biphenylyloxy)alkyl]imidazoles were found to be potent anticonvulsants. The most potent compound of the series, 1-[2-([1,1′-biphenyl]-2-yloxy)ethyl]-1*H*-imidazole (**4**), had an ED_{50} of 15.5 mg/kg against maximal-electroshock-induced seizures in mice after oral administration; the horizontal screen ED_{50} was 320 mg/kg, revealing that the compound has a protective index of 21. Homologues bearing three- and four-carbon tethers between the imidazole and biphenylyloxy moieties were also active, but their potency was attenuated relative to 4. Congeners with the imidazolylalkoxy moiety at the meta or para positions of biphenyl were also less active. All these compounds were potent potentiators of hexobarbital-induced sleeping time in mice, presumably via the well-known imidazole-mediated inhibition of cytochrome P-450. The structural features governing the anticonvulsant and sleeping-time activities appear to be distinct, but a complete dissociation of these two effects has not been achieved. Thus, the potential of these compounds as clinically useful antiepileptic drugs would appear to be limited.

We recently reported the potent and highly selective anticonvulsant activity of α-9*H*-fluoren-2-yl-α-methyl-1*H*-imidazole-1-ethanol (**1**, LY177165) and its congeners.[1] This agent, along with denzimol (**2**) and nafimidone (**3**) (Chart I), is a member of a structurally novel class of anticonvulsants, the (arylalkyl)imidazoles.[2,3] Our previous structure–activity relationship (SAR) studies suggested that the pharmacophore of this class of anticonvulsants is the alkylimidazole portion of the molecule, with the lipophilic aryl portion enabling penetration of the blood–brain barrier.[1]

An impressive feature of the pharmacology of these drugs is their high degree of selectivity; they antagonize maximal electroshock (MES) induced seizures at doses far below those required to produce sedation or neurological impairment but do not antagonize clonic seizures induced by administration of threshold doses of pentylenetetrazole, bicuculline, or picrotoxin.[1,4,5] Although this class of

Chart I



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(1) Robertson, D. W.; Krushinski, J. H.; Beedle, E. E.; Leander, J. D.; Wong, D. T.; Rathbun, R. C. *J. Med. Chem.* **1986**, *29*, 1577.

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