Synthesis and Pharmacology of Some Basic Esters of 3,4,5-Trimethoxybenzoic Acid

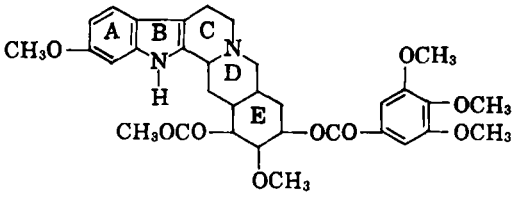
By A. J. VAZAKAS* and JAMES T. DOLUISIO‡

A series of basic esters of 3,4,5-trimethoxybenzoic acid has been synthesized and the compounds evaluated for antihypertensive and local anesthetic activity. One of the compounds, a diamine ester prepared from N-methyl-N-phenyl-N′-propylethylenediamine via a three-step synthesis, exhibited hypotensive activity following intravenous administration to normotensive dogs. No hypotensive activity was demonstrated following intraperitoneal administration to renal hypertensive rats. All but one of the compounds tested exhibited some degree of local anesthetic activity.

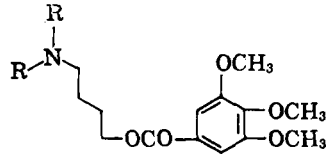
THE ISOLATION of reserpine (I) in crystalline form (1) and the subsequent developments which led to the elucidation of the structure (2–4) and total synthesis (5) of this alkaloid have stimulated many workers to prepare simple analogs of this molecule in search of compounds with reserpine-like activity (6–20).

In our laboratories, a similar objective was approached with the preparation of (*a*) a series of 4-dialkylaminobutyl 3,4,5-trimethoxybenzoates (II), all members of the series possessing portions of rings C, D, and E of the reserpine molecule; (*b*) two 4-(4-substituted piperazino)butyl 3,4,5-trimethoxybenzoates (III) which not only possess portions of rings C, D, and E but, with the inclusion of a second nitrogen atom in a position approximating that of the indole nitrogen of reserpine, might also be considered to possess a portion of ring B; (*c*) N-methyl-N-phenyl-N′-propyl-N′-[4-(3,4,5-trimethoxybenzoyloxy)butyl]-ethylenediamine (IV) which possesses all of ring A and portions of rings B–E.

The compounds of structures II and III were prepared by alkylation of the appropriate secondary amines with the previously unreported 4-chlorobutyl 3,4,5-trimethoxybenzoate (V). For the preparation of IV, N-propylchloroacetamide (VI) was converted by use of N-methylaniline to 2-(N-methylanilino)-N-propylacetamide (VII) which was then reduced with lithium aluminum hydride to N-methyl-N-phenyl-N′-propylethylenediamine (VIII). Acylation of VIII with β-carbethoxypropionyl chloride (IX) afforded the amide ester (X) which, on reduction with lithium aluminum hydride, yielded N-methyl-N-phenyl-N′-propyl-N′-(4-hydroxybutyl)ethylenediamine (XI). The latter was then esterified with 3,4,5-trimethoxybenzoyl chloride in ether and in the presence of triethylamine to produce IV.

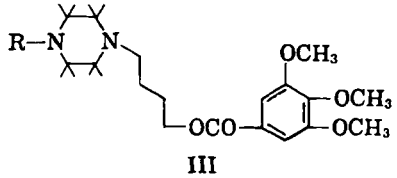


I



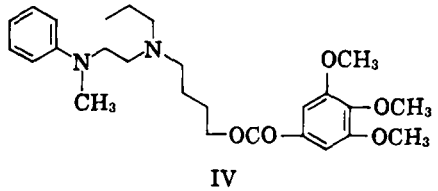
II

IIa, R = CH_3
IIb, R = C_2H_5
IIc, R = n-C_3H_7



III

IIIa, R = CH_3
IIIb, R = $HOCH_2CH_2$



IV

While this work was in progress, a publication

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* Present address: Research Laboratories, National Drug Co., Division of Richardson-Merrell, Inc., Philadelphia, Pa.
‡ Fellow of the American Foundation for Pharmaceutical Education, 1957–1959. Present address: Philadelphia College of Pharmacy and Science, Philadelphia, Pa.

appeared (7) describing the preparation of the compounds of structure II by use of a different synthetic procedure. The compounds were reported to be "devoid of any reserpine-like activity as was indicated by their failure to prolong the hexobarbital sleeping time in mice and their failure to affect the fall-off time of rats from a rotating rod." In our evaluation, the compounds of both structures II and III did not significantly affect the blood pressure of anesthetized normotensive dogs.

Because of the nature of the structures of II and III, it was of interest to examine these compounds also for local anesthetic action. The procedure used consisted of the topical application of 3 drops of a 1% solution of the compound in the rabbit eye and measurement of the duration of anesthesia in minutes. It was found that all of these compounds except III*a* were quite active in this evaluation procedure. The anesthesia produced was accompanied by lacrimation and pitting with each compound except III*b*, which was found to be completely free of these side effects. Structure III*b* also proved to be the most active of the compounds tested. The 87-minute average duration of anesthesia found with III*b* compared favorably with the average durations of action observed for cocaine and procaine of like concentration, these being 54 and 36 minutes, respectively. Another standard, dibucaine, had an average duration of action of 94 minutes at a concentration of 0.5%.

unanesthetized renal hypertensive rats, the compound had no activity when blood pressures were recorded at 1, 2, and 4 hours post treatment at doses of 10 and 20 mg./Kg., i.p. Lack of activity in this experiment may have been due to the short duration of action demonstrated in the dog experiments.

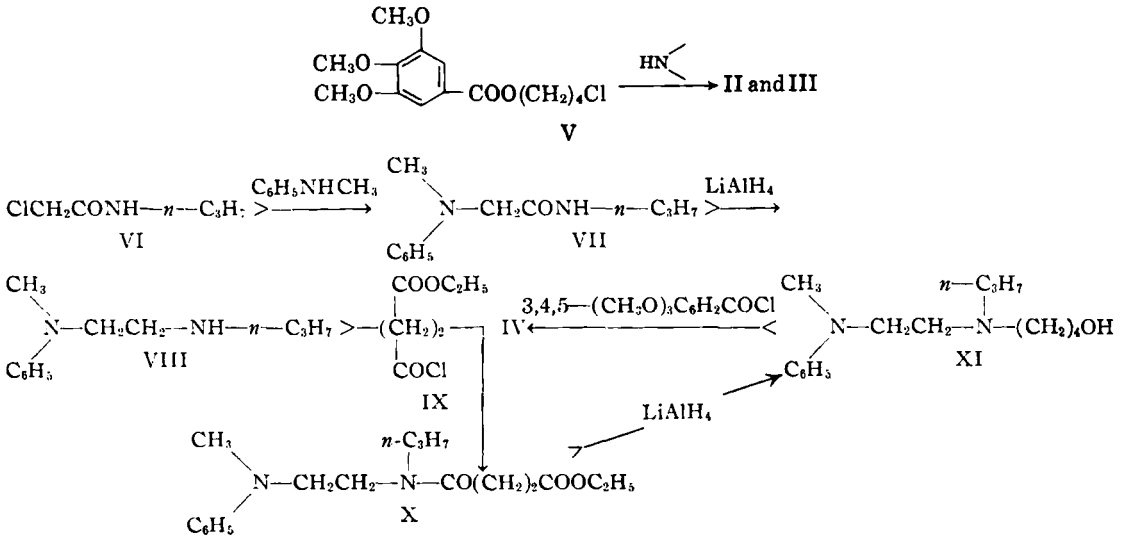
EXPERIMENTAL[1]

3,4,5-Trimethoxybenzoyl Chloride.—This compound, prepared according to the method of Lasslo and Jordan (21), was further purified by distillation *in vacuo*, b.p. 112–115°/0.8 mm. It crystallized in the receiver, m.p. 79.5 to 81°; reported (22) m.p. 79–82°.

4-Chlorobutyl 3,4,5-Trimethoxybenzoate (V).—A mixture of 50.5 Gm. (0.22 mole) of 3,4,5-trimethoxybenzoyl chloride and 29.3 Gm. (0.27 mole) of 4-chloro-1-butanol in 270 ml. of anhydrous benzene was refluxed for 8 hours, hydrogen chloride being evolved. After removal of the solvent, the residue was distilled under reduced pressure. A 42.1-Gm. quantity (63.5%) of pale yellow oil, b.p. 185–189°/1.1 mm. was obtained; n_D^{24} 1.5315.

Anal.—Calcd. for $C_{14}H_{19}ClO_5$: C, 55.54; H, 6.33; Cl, 11.71. Found: C, 55.32; H, 6.33; Cl, 11.71.

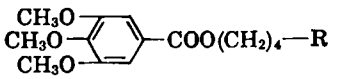
4 - [4 - (2 - Hydroxyethyl)piperazino]butyl 3,4,5-Trimethoxybenzoate Dihydrochloride (IIIb).—A mixture of 5.0 Gm. (0.016 mole) of 4-chlorobutyl 3,4,5-trimethoxybenzoate, 2.1 Gm. (0.016 mole) of N-(2-hydroxyethyl)piperazine, 1.7 Gm. of anhydrous sodium carbonate, and 3 Gm. of powdered potassium iodide in 200 ml. of methyl ethyl ketone was stirred and refluxed for 16 hours. The solvent was removed at reduced pressure, the residue treated with a concentrated solution of sodium carbonate, and the alkaline mixture extracted with ether. The dihydrochloride precipitated upon the addition of anhydrous hydrogen chloride to the dried ether solution. The white solid was collected, washed with ether, and recrystallized from ethanol to

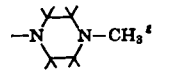
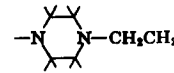


Compound IV, which was evaluated only with regard to its effect on blood pressure, had hypotensive activity when administered to anesthetized normotensive dogs at a dose of 8 mg./Kg., i.v. This dose caused an immediate fall in blood pressure ranging from 41 to 68 mm. of mercury in three dogs. The average duration of activity was approximately 17 minutes, the range being from 5 to 30 minutes. The immediate decrease in blood pressure observed with this compound is not typical of reserpine activity. The compound did not appear to possess adrenergic or ganglionic blocking activity. In

[1] Melting points were determined by the open capillary tube method and are uncorrected. The microanalyses were done by Dr. Alfred Bernhardt, Mülheim (Ruhr), Germany. Spectra of the oils were obtained on a Perkin-Elmer model 21A double beam infrared spectrophotometer on samples between salt disks.

TABLE I.—BASIC ESTERS OF 3,4,5-TRIMETHOXYBENZOIC ACID



No.	R	M.p., °C.	Yield, %	Formula	C, % Calcd.	C, % Found	H, % Calcd.	H, % Found	Cl, % Calcd.	Cl, % Found	N, % Calcd.	N, % Found
1	$-N(CH_3)_2$[a]	121.5 to 122[b]	39	$C_{18}H_{25}NO_5 \cdot HCl$	55.24	55.24	7.53	7.47	...	...	4.03	4.22
2	$-N(C_2H_5)_2$[c]	138.5 to 139.5[d]	35	$C_{18}H_{29}NO_5 \cdot HCl$	57.51	57.24	8.04	8.16	...	...	3.73	3.97
3	$-N(n-C_3H_7)_2$[e]	118–119[f]	25	$C_{20}H_{33}NO_5 \cdot HCl$	59.46	59.36	8.48	8.13	...	...	3.47	3.66
4		207[h]	45	$C_{19}H_{30}N_2O_5 \cdot 2HCl$	...	...	...	...	16.14	15.99	6.38	6.28
5		190[h]	56	$C_{20}H_{32}N_2O_6 \cdot 2HCl$	...	...	...	...	15.11	14.90	5.97	5.92

[a] Clusters of small white needles from benzene. [b] Reported (7) m.p. 122–124°. [c] White needles from acetone-ether. [d] Reported (7) m.p. 140–141°. [e] Rosettes of white needles from acetone-ether. [f] Reported (7) m.p. 118–119°. [g] Mats of white needles from ethanol or from a large volume of acetone. [h] With decomposition. [i] Rhombs from ethanol.

yield 4.2 Gm. (56%) of white crystalline product, m.p. 190° dec.

Anal.—Calcd. for $C_{20}H_{32}N_2O_6 \cdot 2HCl$: Cl, 15.11; N, 5.97. Found: Cl, 14.90; N, 5.92.

The compounds listed in Table I were prepared essentially as above, the only variations in the procedure being (*a*) the use of excess amine, rather than sodium carbonate, as the acid acceptor in the preparation of Nos. *1–3*, and (*b*) the preparation of No. *1* in a pressure bottle, rather than under reflux, at a temperature of 60° for 30 hours.

N-Propylchloroacetamide (VI).—This compound, prepared by the method reported for the synthesis of the N-ethyl homolog (23), was obtained as a colorless oil in 75% yield, b.p. 91–94°/4.5 mm.; reported (24) b.p. 90–91°/4 mm.

2-(N-Methylanilino)-N-propylacetamide (VII).—A mixture of 102 Gm. (0.75 mole) of N-propylchloroacetamide and 181 Gm. (1.7 moles) of N-methylaniline in 700 ml. of toluene was stirred and refluxed for 8 hours. The mixture, after having been allowed to stand overnight, was filtered to remove the precipitated N-methylaniline hydrochloride, the cake was washed with benzene, and the benzene-toluene filtrate was evaporated to dryness under vacuum with the aid of steam bath heat. The dark oily residue was dissolved in chloroform, the chloroform solution was washed with water, then dried over sodium sulfate. The mixture was filtered, the chloroform removed, and the residual oil distilled *in vacuo*. After a small forerun of N-methylaniline, 131 Gm. of oily product was obtained, b.p. 135–138°/0.4 to 0.5 mm., which gradually solidified in the receiver, forming rosettes of glistening pale-yellow needles. The product was further purified by recrystallization from 2 L. of skelly B. It crystallized in large shiny white plates. The yield was 118 Gm. (76%), m.p. 52–53°.

Anal.—Calcd. for $C_{12}H_{18}N_2O$: N, 13.58. Found: N, 13.64.

N-Methyl-N-phenyl-N′-propylethylenediamine (VIII).—A 20.0 Gm. (0.526 mole) quantity of lithium aluminum hydride was added, portionwise and with stirring, to 500 ml. of tetrahydrofuran under nitrogen. After addition was complete, the mixture was stirred for 30 minutes. A solution of 36.0 Gm. (0.175 mole) of 2-(N-methylanilino)-N-propylacetamide in 150 ml. of tetrahydrofuran was then added, dropwise, to the stirred mixture, the addition requiring approximately 30 minutes for completion. The reaction mixture became warm but did not reflux. The mixture was stirred for 30 minutes, then heat was applied; it was refluxed with stirring for 38 hours.[a] A 100-ml. quantity of a 20% solution of potassium sodium tartrate was then added, dropwise and with stirring, to the cold (ice bath) mixture. The resulting white precipitate was removed by suction filtration, the cake was washed with 200 ml. of hot tetrahydrofuran, and the

[a] This 38-hour period of reflux was selected arbitrarily and should not be considered a minimum requirement for this reaction. It has been observed (25) that the reduction of N-monosubstituted amides requires not only a considerable excess of hydride but also a fairly long period of reflux after the addition of the amide has been completed if good yields are to be obtained. We repeated this preparation with reactants, solvent, and procedure remaining the same, but with the reflux period limited to 2.5 hours, a reflux period which we have found adequate for the reduction of N-unsubstituted amides in good yield. In these experiments, although the mole ratio of hydride:amide was varied between 2:1 and 4:1, a 2.5 hour reflux period gave, in all cases, yields between 18 and 20% of VIII.

combined pale-yellow filtrates were dried over sodium sulfate and filtered. The solvent was removed at reduced pressure and, after the last traces of moisture had been removed by co-distillation with benzene, the residue was fractionated through a short Vigreux column to yield 24.4 Gm. (72.6%) of very fluid, pale greenish-yellow oil, b.p. 107–109°/1.0 mm.; n_D^{25} 1.5331.

Anal.—Calcd. for $C_{12}H_{20}N_2$: C, 74.95; H, 10.48; N, 14.57. Found: C, 74.87; H, 10.63; N, 14.51.

β-Carbethoxypropionyl Chloride (IX).—This compound was prepared from β-carbethoxypropionic acid by the procedure of Riegel and Lilienfeld (26) in 78% yield, b.p. 109–110°/28 mm.; reported (26) b.p. 110–115°/30 mm. The β-carbethoxypropionic acid, prepared from succinic anhydride and ethanol as reported (26) and further purified by distillation, was obtained in 47% yield, b.p. 118–120°/3 mm.; reported (27) b.p. 119°/3 mm.

N - (2 - N - Methylanilinoethyl) - N - propyl - β-carbethoxypropionamide (X).—A solution of 9.87 Gm. (0.060 mole) of β-carbethoxypropionyl chloride in 50 ml. of benzene was added, dropwise and with stirring, to a well cooled solution of 23.8 Gm. (0.124 mole) of N-methyl-N-phenyl-N′-propylethylenediamine in 100 ml. of benzene. When addition was complete, the reaction mixture was stirred at room temperature for 1 hour, then refluxed for 2 hours. The mixture was cooled, water was added with stirring to dissolve the copious white crystalline precipitate which had formed, and, while being stirred vigorously, the two-phase system was acidified with dilute hydrochloric acid. The aqueous phase was separated, washed well with ether, then made basic with a saturated solution of sodium carbonate. The basic mixture was extracted with ether, the ether solution was dried (sodium sulfate), filtered, and the ether removed by distillation under vacuum. The residual oil, after the last traces of moisture had been removed by co-distillation with benzene, was fractionated through a short Vigreux column. After removal of 11.8 Gm. of the starting diamine, which had also served as acid acceptor, in the forerun, 11.6 Gm. (60%) of viscous, pale-yellow oil was obtained, b.p. 166–168°/0.1 mm.; n_D^{25} 1.5240.

Infrared spectrum: ester, 1740 cm.$^{-1}$, 1200 cm.$^{-1}$ (broad); amide, 1640 cm.$^{-1}$

Anal.—Calcd. for $C_{18}H_{28}N_2O_3$: C, 67.47; H, 8.81; N, 8.74. Found: C, 67.22; H, 8.69; N, 8.89.

N - Methyl - N - phenyl - N′ - propyl - N′ - (4-hydroxybutyl)ethylenediamine (XI).—This compound was prepared by the lithium aluminum hydride reduction of N-(2-N-methylanilinoethyl)-N-propyl-β-carbethoxypropionamide (X) by the same method used for the preparation of N-methyl-N-phenyl-N′-propylethylenediamine (VIII). In this reduction, a solution of 10.0 Gm. (0.031 mole) of the amide-ester (X) in 40 ml. of tetrahydrofuran was added to a mixture of 4.72 Gm. (0.124 mole) of lithium aluminum hydride in 100 ml. of tetrahydrofuran under nitrogen. The duration of reflux was 11 hours, and 35 ml. of 20% potassium sodium tartrate was used for the subsequent hydrolysis of complex and of excess hydride. Fractionation gave 7.13 Gm. (86%) of pale-yellow oil, b.p. 132–133°/0.1 mm.; n_D^{25} 1.5301.

Infrared spectrum: O—H stretch, 3330 cm.$^{-1}$; primary alcohol, 1070 cm.$^{-1}$; ester and amide, absent.

Anal.—Calcd. for $C_{16}H_{28}N_2O$: C, 72.68; H, 10.67; N, 10.60. Found: C, 72.57; H, 10.42; N, 10.52.

N - Methyl - N - phenyl - N′ - propyl - N′ - [4 - (3,4,5 - trimethoxybenzoyloxy)butyl]ethylenediamine (IV).—To a solution of 5.53 Gm. (0.024 mole) of 3,4,5-trimethoxybenzoyl chloride in 100 ml. of anhydrous ether, a solution of 6.0 Gm. (0.06 mole) of triethylamine in 20 ml. of anhydrous ether was added dropwise and with stirring. To the white silky suspension of the complex which had formed, a solution of 5.28 Gm. (0.020 mole) of N-methyl-N-phenyl-N′-propyl-N′-(4-hydroxybutyl)ethylenediamine (XI) in 50 ml. of anhydrous ether was added, dropwise and with stirring. The mixture was stirred at room temperature for 2 hours, then stirred and refluxed for 3 hours. The copious white crystalline precipitate of triethylamine hydrochloride which had formed was removed by suction filtration, the cake washed with ether, and the ether and excess triethylamine removed from the combined filtrates by vacuum distillation to leave a pale-yellow oily residue. The oily product was dissolved in ether and extracted with dilute hydrochloric acid. The acidic aqueous solution was washed twice with ether, then made basic with a concentrated solution of sodium carbonate. The insoluble oily product was taken up in ether, the ether solution dried (sodium sulfate), filtered, the solvent removed to dryness, and traces of moisture remaining removed by co-distillation with benzene. The product, which began to decompose on attempted distillation *in vacuo*, was distilled successfully by use of an Asco "50" rota-film molecular still at a jacket temperature of 240–242°/27–30μ to yield 5.28 Gm. (57.6%) of very viscous pale-yellow oil; n_D^{25} 1.5495.

Infrared spectrum: ester, 1717 cm.$^{-1}$, 1222 cm.$^{-1}$; ether, 1123 cm.$^{-1}$

Anal.—Calcd. for $C_{26}H_{38}N_2O_5$: C, 68.09; H, 8.35; N, 6.11. Found: C, 67.72; H, 8.16; N, 6.03.

Attempts to prepare the crystalline hydrochloride, maleate, and *d*-tartrate of this compound all proved unsuccessful.

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Hemolysis of Erythrocytes by Antibacterial Preservatives

By H. C. ANSEL and D. E. CADWALLADER

A study has been made of the hemolytic activity of antibacterial preservatives on human and rabbit erythrocytes in the presence of 0.9 per cent sodium chloride. The hemolytic potency of phenol increased with methyl and/or chlorine substitution. The order of activity was: *p*-chloro-*m*-cresol ⟩ *p*-chlorophenol ⟩ tricresol and *m*-cresol ⟩ phenol. Chlorinated alcohols were similarly more hemolytic than the non-halogenated ones: *p*-chloro-β-phenylethyl alcohol ⟩ chlorobutanol ⟩ phenylethyl alcohol ⟩ benzyl alcohol. Erythrocytes were highly sensitive to benzalkonium chloride, benzethonium chloride, and phenylmercuric nitrate. Thimerosal, butylparaben, and sodium formaldehyde sulfoxylate also induced hemolysis. The data indicate that the hemolytic and antimicrobial mechanisms of action may be identical for many compounds.

THE U.S.P. XVI (1) states that suitable substances may be added to preparations intended for parenteral administration to increase the stability of the product, provided they are harmless in the amounts administered and do not interfere with the therapeutic efficacy or the assay procedures. Limited amounts of various antibacterial preservatives are recommended for multiple-dose parenteral products regardless of the method of sterilization. A personal survey of currently marketed parenteral products indicated that a variety of antibacterial agents are used in preparations intended for each route of administration, including intravenous administration.

It has been shown, *in vitro* and *in vivo*, that certain chemicals in amounts calculated to be isoosmotic to the red blood cell according to physicochemical data may pass through the erythrocytic membrane and fail to prevent osmotic hemolysis (2–9). In addition, certain substances may be cytotoxic to the erythrocyte, thus altering the integrity of the membrane and resulting in the escape and/or denaturation of the cell contents.

It was the purpose of this investigation to study the hemolytic activity of various antibacterial preservatives on human and rabbit erythrocytes in the presence of isotonic media. By preparing the preservative solutions in 0.9% sodium chloride it was felt that any alteration in the integrity of the erythrocytes could be directly attributed to the action of the preservative and not to the original osmotic pressure of the external solution.

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EXPERIMENTAL

Collection of Blood.—The rabbit and human (Caucasian) blood samples employed in this investigation were obtained in the manner described by Grosicki and Husa (9). Caucasian blood was consistently employed to avoid the osmotic differences found in blood samples from Negro donors (8). Fresh blood samples from healthy donors were used throughout the study.

Materials.—The following chemicals used in this study were supplied gratuitously by their respective manufacturers: chlorobutanol U.S.P. and benzyl alcohol N.F., Benzol Products Co.; methylparaben U.S.P., propylparaben U.S.P., ethylparaben, and butylparaben, Heyden Newport Chemical Corp.; phenylmercuric nitrate, Metalsalts Corp.; *p*-chlorophenol, Dow Chemical Co.; *p*-chloro-*m*-cresol, Burroughs Wellcome and Co.; thimerosal N.F., Eli Lilly and Co.; sodium formaldehyde sulfoxylate, Rohm and Haas; and *p*-chloro-β-phenylethyl