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Quaternary Salts of Substituted 2-Aminoethyl *N*-Benzoylaminobenzoate. A New Class of Smooth Muscle Relaxant Agents

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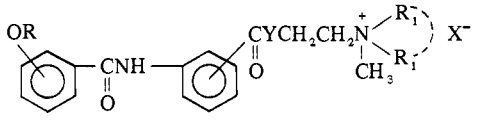
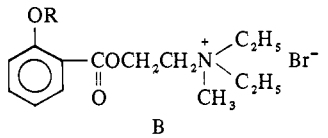
We have described previously[1] the synthesis and smooth muscle relaxant properties of some *N*-(dialkylaminoalkyl) *p*-(2-alkoxybenzamido)benzoate quaternary salts. In order to gain further insight on structure-activity relationships, we have synthesized several other compounds referable to the general formulas A and B.



A



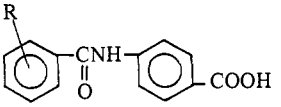
B

Chemistry. The above-mentioned compounds were synthesized from the corresponding bases by quaternization with methyl halides. The bases were obtained by condensation of the appropriate acyl chloride with N-disubstituted 2-aminoethanol (method A) or by reaction of 2-(diethylamino)ethyl *p*-aminobenzoate with the appropriate substituted benzoyl chloride (method B). The new substituted benzoylaminobenzoic acids were prepared by acylation of aminobenzoic acid with the appropriate substituted benzoyl chloride. The characteristics of the new compounds are reported in Tables I-IV.

Pharmacology. The data related to smooth muscle relaxant activities are reported in Table V. It will be noted that, among the compounds tested, **39**, **42**, and **43** were shown to possess similar anticholinergic activity but considerably greater barium-induced contraction antagonizing properties as compared to scopolamine *N*-butyl bromide. Structural modifications of one of the most effective compounds, namely **42**, led to the synthesis of several derivatives, **40**, **41**, **44-48**, and **59**, whose effectiveness was inferior to that of the parent compound.

Quaternization of the same base with either MeI or MeBr resulted in compounds of comparable biological potency. Quaternization is accompanied by (a) increased toxicity; (b) increased anticholinergic properties; and (c) as a rule, decreased or increased effectiveness on histamine- and $BaCl_2$-induced spasm proportional to side-chain length in the R position. As far as some effects of quaternization are concerned (a and b), our results are in agreement with data in the literature.[2,3] Unlike toxicity, smooth muscle relaxant properties seem to be dependent upon side-chain length in the R position.

Table I



No.	R	% yield	Mp, °C	Formula[a]
1[b]	2-OCH_3	91	243-244	$C_{15}H_{13}NO_4$
2[b]	2-OC_2H_5	92	237-238	$C_{16}H_{15}NO_4$
3[b]	2-*n*-OC_4H_9	88	196-198	$C_{18}H_{19}NO_4$
4	2-*n*-OC_5H_{11}	89	187-189	$C_{19}H_{21}NO_4$
5	2-*n*-OC_8H_{17}	92	172-174	$C_{22}H_{27}NO_4$
6	3-*n*-OC_8H_{17}	85	220-221	$C_{22}H_{27}NO_4$
7	4-*n*-OC_8H_{17}	84	265-267	$C_{22}H_{27}NO_4$
8	2-$OCH_2C_6H_5$	88	211-212	$C_{21}H_{17}NO_4$
9	2-$O(CH_2)_2C_6H_5$	88	209-211	$C_{22}H_{19}NO_4$
10	2-$O(CH_2)_3C_6H_5$	90	196-198	$C_{23}H_{21}NO_4$

[a] All compounds were analyzed for C, H, and N. [b] Known; M. Ghelardoni and F. Russo, Belgian Patent 670,751 (1966); *Chem. Abstr.*, 65, 16909*d* (1966).

Experimental Section

Chemical Procedures. Melting points were determined in an open capillary tube in a bath and are uncorrected. Ir spectra of the compounds in Nujol mulls were determined by means of a Perkin-Elmer 337 grating spectrometer. All spectra were consistent with the assigned structures.

Preparation of Substituted Benzoylaminobenzoic Acids (1-10; See Table I). These were made by refluxing the corresponding acid chloride with *p*-aminobenzoic acid in dioxane and pyridine for 2 hr. The precipitate was collected and extracted with 5% $NaHCO_3$. The solution was then acidified and the precipitate was filtered off and crystallized from EtOH.

Substituted 2-Aminoethyl *p*-Aminobenzoate (11-23, 25-30; See Table II). Method A (11, 12, 15, 16, 19-24, 28-30). The appropriate substituted *p*-benzamidobenzoic acid in $SOCl_2$ was refluxed gently for 2 hr. The excess $SOCl_2$ was removed under reduced pressure. The obtained acid chloride was added slowly with stirring to a solution of N-disubstituted 2-aminoethanol in pyridine. The mixture was stirred for 3 hr at 100°, cooled, and poured into H_2O. The solid was washed with H_2O and then crystallized.

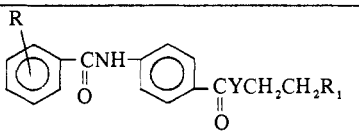
Method B (13, 14, 17, 18, 25-27). A solution of 2-(diethylamino)ethyl *p*-aminobenzoate (0.1 mol) in H_2O (100 ml) was added slowly with stirring to a solution of 0.1 mol of the appropriate substituted benzoyl chloride. The mixture was maintained alkaline with 10% NaOH. The precipitate was collected and crystallized. When an oil was obtained, it was changed to the solid hydrochloride.

1-[*N*-(2-Octyloxybenzoyl)-*p*-aminobenzoyl]-4-diethylethylenediamine (24; See Table II). Method A was followed except that 2-diethylaminoethylamine was used, instead of N-disubstituted 2-aminoethanol.

Quaternary Salts 31-55 (Table III) and 57-59 (Table IV). These were prepared by dissolution of the corresponding bases in Et_2O followed by treating with MeI (or MeBr) at room temperature. The precipitate was removed by filtration and recrystallized.

2-(Diethylamino)ethyl (*o*-Octyloxy)benzoate (58; Table IV). Method A was followed except that (*o*-octyloxy)benzoyl chloride

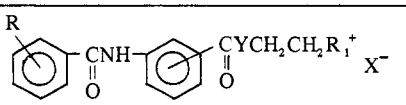
Table II



No.	R	Y	R_1	% yield	Mp, °C	Crystals from	Formula[a]
11[b]	2-OCH_3	O	$N(C_2H_5)_2$	80	57-59	Benzene	$C_{21}H_{26}N_2O_4$
12[b]	2-OC_2H_5	O	$N(C_2H_5)_2$	83	94-96	EtOH	$C_{22}H_{28}N_2O_4$
13	2-$OCH_2CH{=}CH_2$	O	$N(C_2H_5)_2$	68	81-83	Hexane	$C_{23}H_{28}N_2O_4$
14	2-*n*-OC_3H_7	O	$N(C_2H_5)_2 \cdot HCl$	76	183-185	Dioxane	$C_{23}H_{30}N_2O_4 \cdot HCl$
15[b]	2-*n*-OC_4H_9	O	$N(C_2H_5)_2$	80	81-82	Hexane	$C_{24}H_{32}N_2O_4$
16[b]	2-*n*-OC_5H_{11}	O	$N(C_2H_5)_2$	78	70-72	Hexane	$C_{25}H_{34}N_2O_4$
17	2-$OCH(C_2H_5)_2$	O	$N(C_2H_5)_2 \cdot HCl$	71	111-113	Benzene	$C_{25}H_{34}N_2O_4 \cdot HCl$
18	2-*n*-OC_7H_{15}	O	$N(C_2H_5)_2$	73	61-63	Hexane	$C_{27}H_{38}N_2O_4$
19[b]	2-*n*-OC_8H_{17}	O	$N(C_2H_5)_2 \cdot HCl$	81	137-139	Benzene	$C_{28}H_{40}N_2O_4 \cdot HCl$
20	2-*n*-OC_8H_{17}	O	$N(CH_2)_4CH_2$	84	70-72	Hexane	$C_{29}H_{40}N_2O_4$
21	2-*n*-OC_8H_{17}	O	$N(CH_2)_2OCH_2CH_2$	80	95-97	EtOH	$C_{28}H_{38}N_2O_5$
22	3-*n*-OC_8H_{17}	O	$N(C_2H_5)_2 \cdot HCl$	75	83-85	EtOH	$C_{28}H_{40}N_2O_4 \cdot HCl$
23	4-*n*-OC_8H_{17}	O	$N(C_2H_5)_2$	78	119-121	Me_2CO	$C_{28}H_{40}N_2O_4$
24	2-*n*-OC_8H_{17}	NH	$N(C_2H_5)_2$	85	124-126	Dioxane	$C_{28}H_{41}N_3O_3$
25[b]	2-*n*-OC_9H_{19}	O	$N(C_2H_5)_2 \cdot HCl$	70	127-129	Benzene	$C_{29}H_{42}N_2O_4 \cdot HCl$
26[b]	2-*n*-$OC_{10}H_{21}$	O	$N(C_2H_5)_2 \cdot HCl$	72	132-134	Benzene	$C_{30}H_{44}N_2O_4 \cdot HCl$
27[b]	2-*n*-$OC_{12}H_{25}$	O	$N(C_2H_5)_2 \cdot HCl$	75	140-142	Benzene	$C_{32}H_{48}N_2O_4 \cdot HCl$
28	2-$OCH_2C_6H_5$	O	$N(C_2H_5)_2 \cdot HCl$	80	175-176	Dioxane	$C_{27}H_{30}N_2O_4 \cdot HCl$
29	2-$O(CH_2)_2C_6H_5$	O	$N(C_2H_5)_2$	82	105-107	Cyclohexane	$C_{28}H_{32}N_2O_4$
30	2-$O(CH_2)_3C_6H_5$	O	$N(C_2H_5)_2$	82	66-68	Hexane	$C_{29}H_{34}N_2O_4$

[a]All compounds were analyzed for C, H, and N. [b]Known, see ref 1.

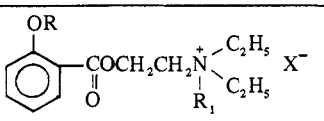
Table III



No.	R	$-COY(CH_2)_2R_1^+$ position	Y	R_1^+	X^-	% yield	Crystals from	Mp, °C	Formula[a]
31[b]	2-OCH_3	4	O	$NCH_3(C_2H_5)_2$	I	93	EtOH	198-200	$C_{22}H_{29}IN_2O_4$
32	2-OC_2H_5	4	O	$NCH_3(C_2H_5)_2$	Br	95	EtOH	191-193	$C_{23}H_{31}BrN_2O_4$
33[b]	2-OC_2H_5	4	O	$NCH_3(C_2H_5)_2$	I	92	EtOH	200 dec	$C_{23}H_{31}IN_2O_4$
34	2-*n*-OC_3H_7	4	O	$NCH_3(C_2H_5)_2$	Br	90	Me_2CO	178-180	$C_{24}H_{33}BrN_2O_4$
35	2-$OCH_2CH{=}CH_2$	4	O	$NCH_3(C_2H_5)_2$	Br	89	EtOH	186-188	$C_{24}H_{31}BrN_2O_4$
36[b]	2-*n*-OC_4H_9	4	O	$NCH_3(C_2H_5)_2$	I	87	EtOH	155-157	$C_{25}H_{35}IN_2O_4$
37[b]	2-*n*-OC_5H_{11}	4	O	$NCH_3(C_2H_5)_2$	I	91	H_2O	163-166	$C_{26}H_{37}IN_2O_4$
38	2-$OCH(C_2H_5)_2$	4	O	$NCH_3(C_2H_5)_2$	Br	90	AcOEt	138-140	$C_{26}H_{37}BrN_2O_4$
39	2-*n*-OC_7H_{15}	4	O	$NCH_3(C_2H_5)_2$	Br	92	*i*-PrOH	168-170	$C_{28}H_{41}BrN_2O_4$
40	2-*n*-OC_8H_{17}	2	O	$NCH_3(C_2H_5)_2$	Br	94	AcOEt	70-72	$C_{29}H_{43}BrN_2O_4$
41	2-*n*-OC_8H_{17}	3	O	$NCH_3(C_2H_5)_2$	Br	94	Dioxane	116-118	$C_{29}H_{43}BrN_2O_4$
42[b]	2-*n*-OC_8H_{17}	4	O	$NCH_3(C_2H_5)_2$	Br	95	EtOH	166-168	$C_{29}H_{43}BrN_2O_4$
43[b]	2-*n*-OC_8H_{17}	4	O	$NCH_3(C_2H_5)_2$	I	91	Me_2CO	136-138	$C_{29}H_{43}IN_2O_4$
44	2-*n*-OC_8H_{17}	4	O	$H_3CN(CH_2)_4CH_2$	Br	93	EtOH-Et_2O	146-148	$C_{30}H_{43}BrN_2O_4$
45	2-*n*-OC_8H_{17}	4	O	$H_3CN(CH_2)_2OCH_2CH_2$	Br	90	*i*-PrOH	137-139	$C_{29}H_{41}BrN_2O_5$
46	3-*n*-OC_8H_{17}	4	O	$NCH_3(C_2H_5)_2$	Br	90	EtOH-Et_2O	128-130	$C_{29}H_{43}BrN_2O_4$
47	4-*n*-OC_8H_{17}	4	O	$NCH_3(C_2H_5)_2$	Br	86	AcOEt	158-160	$C_{29}H_{43}BrN_2O_4$
48	2-*n*-OC_8H_{17}	4	NH	$NCH_3(C_2H_5)_2$	Br	88	*i*-PrOH	147-149	$C_{29}H_{44}BrN_3O_3$
49[b]	2-*n*-OC_9H_{19}	4	O	$NCH_3(C_2H_5)_2$	Br	91	Me_2CO	147-149	$C_{30}H_{45}BrN_2O_4$
50[b]	2-*n*-OC_9H_{19}	4	O	$NCH_3(C_2H_5)_2$	I	92	H_2O	127-130	$C_{30}H_{45}IN_2O_4$
51[b]	2-*n*-$OC_{10}H_{21}$	4	O	$NCH_3(C_2H_5)_2$	I	90	H_2O	115-117	$C_{31}H_{47}IN_2O_4$
52[b]	2-*n*-$OC_{12}H_{25}$	4	O	$NCH_3(C_2H_5)_2$	I	91	Me_2CO-Et_2O	81-83	$C_{33}H_{51}IN_2O_4$
53	2-$OCH_2C_6H_5$	4	O	$NCH_3(C_2H_5)_2$	Br	92	Me_2CO	186-188	$C_{28}H_{33}BrN_2O_4$
54	2-$O(CH_2)_2C_6H_5$	4	O	$NCH_3(C_2H_5)_2$	Br	87	*i*-PrOH	164-166	$C_{29}H_{35}BrN_2O_4$
55	2-$O(CH_2)_3C_6H_5$	4	O	$NCH_3(C_2H_5)_2$	Br	89	*i*-PrOH	165-167	$C_{30}H_{37}BrN_2O_4$

[a]All compounds were analyzed for C, H, and N. [b]Known, see ref 1.

Table IV



No.	R	R_1	X^-	Crystals from	% yield	Mp, °C	Formula[a]
56[b]	C_2H_5	H	Cl	*i*-PrOH	85	138-140	$C_{15}H_{23}NO_3 \cdot HCl$
57	C_2H_5	CH_3	Br	AcOEt	92	91-93	$C_{16}H_{26}BrNO_3$
58	*n*-C_8H_{17}	H	Cl	Me_2CO	85	131-133	$C_{21}H_{35}NO_3 \cdot HCl$
59	*n*-C_8H_{17}	CH_3	Br	*i*-PrOH	95	72-74	$C_{22}H_{38}BrNO_3$

[a]All compounds were analyzed for C, H, and N. [b]Known; J. Thomas and J. Canty, *J. Pharm. Pharmacol.*, 14, 587 (1962).

Table V. Pharmacological Screening Results

No.	LD_{50} (mouse), mg/kg ip	Smooth muscle relaxant activity *in vitro* (guinea-pig ileum), ED_{50} in μg/ml: Preventive activity against Acetylcholine, 1×10^{-8}	Preventive activity against Histamine, 1×10^{-8}	Preventive activity against Barium chloride, 1×10^{-4}	Curative activity against acetylcholine, 1×10^{-8}	Charcoal meal test (rat), ED_{50} in mg/kg ip
11	168.0	2.4	5.3	9.9	5.0	>40.0
12	363.0	1.4	2.7	7.7	3.8	18.8
15	300.0	1.6	2.4	6.3	0.8	9.2
16	346.0	0.7	6.3	37.6	1.6	7.5
19	501.0					7.9
25		>51.9	>51.9	>51.9	36.3	18.8
26	>400.0	>53.3	>53.3	>53.3	>53.3	10.9
27	>300.0[a]	>22.4	>56.2	>22.4	>22.4	6.6
31	74.0	4.0			6.8	24.1
32	126.0	0.8	11.6	24.2	0.6	9.9
33	149.0	0.8	13.2	37.6	0.5	10.5
34	125.0	0.9	7.2	9.9	1.0	11.1
35	123.0	0.8	15.1	31.2	0.6	7.1
36	169.0	0.6	6.7	7.5	0.6	7.9
37	>150.0[a]	0.3	2.4	2.9	0.1	6.9
38	163.0	0.1	1.3	5.3	0.2	4.5
39	79.0	0.06	0.6	1.2	0.05	5.0
40	44.6	0.5	8.4	5.6	0.5	2.3
41	81.4	0.7	8.6	11.2	1.2	4.4
42	87.0	0.04	0.5	0.9	0.02	1.8
43	65.6	0.02	0.9	1.9	0.05	1.6
44	70.0	0.2	1.0	3.4	0.1	
45	57.5	0.07	1.4	7.5	0.1	
46	61.0	0.01	16.8	20.2	0.1	3.0
47	<30.0	0.03	3.2	>22.4	0.09	3.3
48	87.0	0.03	4.7	4.5	0.04	1.7
49	71.6	0.03	0.7	1.4	0.1	1.8
50	88.0	0.04	0.1	0.9	0.1	2.5
51	96.6	0.05	3.4	6.4	0.2	2.2
52	151.0	0.5	2.7	>26.6	0.6	3.2
53	50-100	0.1	3.3	10.8	0.2	4.9
54	125.5	0.6	2.1	4.3	0.3	4.4
55	166.0	0.2	2.2	7.7	0.2	3.2
57	115.0	0.29	6.85		0.20	
59	70.0	0.04		5.1	0.04	
Papaverine HCl	108.0	2.7	2.5	5.1	1.7	>40.0
Scopolamine *N*-butyl bromide	72.5	0.02	83.1	77.6	0.02	11.3

[a] Water suspension.

was used instead of substituted benzoyl-*p*-aminobenzoic acid chloride.

Pharmacological Methods. Quaternary derivatives are readily soluble in water whereas the bases were solubilized by adding stoichiometric amounts of HCl.

(a) Acute Toxicity. Mice, Swiss-Morini strain, weighing 17-22 g, were used. The compounds were administered intraperitoneally to groups of ten mice per dose level. The animals were kept under observation for a period of 10 days. LD_{50} was calculated according to Litchfield and Wilcoxon.[4]

(b) *In Vitro* Smooth Muscle Relaxant Activity. The isolated guinea-pig ileum was used as a test model.[5] Compounds to be tested were added to the bathing solution either 1 min before the addition of the spasm-inducing agent or at the time of the spastic agent-induced maximal contraction. The potential inhibitory effects of each compound were determined on not less than four segments of ileum per dose level. ED_{50} was calculated according to Litchfield and Wilcoxon.[4]

(c) Charcoal Meal Test. This test measures inhibition or stimulation of peristalsis[6,7] and it is considered a useful tool to determine the effect of anticholinergic compounds.[8] Rats, Wistar-Morini strain, weighing 160–220 g, were used. After an overnight fasting, the animals received by gavage 25 ml/kg of a 10% charcoal suspension in 10% gum acacia. Thirty minutes after, the animals were killed by a blow on the head and the small intestine was removed. The per cent of the small intestine which had been traversed by the charcoal was then recorded. Compounds to be tested were injected intraperitoneally 15 min before charcoal meal to groups of ten rats each per dose level. For each compound an ED_{50} was determined[4] which represents the approximate dose required to produce an average 50% inhibition of propulsion.

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