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SYNTHESIS AND PHARMACOLOGICAL INVESTIGATION OF NEW ALKOXYBENZAMIDES—I

3:4:5-TRIMETHOXYBENZAMIDES

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Abstract—Some new 3:4:5-trimethoxybenzoic acid amides formed particularly with heterocyclic bases have been prepared and examined for toxicity, tranquillizing action and analgesic activity. 3:4:5-Trimethoxybenzoyl morpholine and its 2-methyl derivative displayed considerable tranquillizing and analgesic effects together with slight toxicity.

THE observation that a number of biologically active compounds occurring in nature contained trimethoxyphenyl or trimethoxybenzoyl groups as a part of their molecule (e.g. the alkaloids of Rauwolfia, colchicine, podophyllotoxin, mescaline), prompted us a few years ago to start some systematic investigations in the field of this group of compounds. Since several papers of other authors, dealing with related materials, have been published in the meantime,[1] it appears to us reasonable now to give an account of our own researches.

From among some simple compounds tested pharmacologically by us, 3:4:5-trimethoxybenzamide was found to possess a weak tranquillizing action; this fact directed our work to aim at the synthesis of various amides of alkoxybenzoic acids, primarily those of 3:4:5:-trimethoxybenzoic acid, with a view to developing therapeutically useful products.

Table 1 summarizes the synthesized 3:4:5-trimethoxy-benzoyl amides, together with some of their most important properties, which had not been reported previously in the literature. The general method of synthesizing these compounds was to react 3:4:5-trimethoxybenzoyl chloride (prepared conveniently from the acid and thionyl chloride) in benzene solution with the corresponding amine, in the presence of triethylamine.

The bases used as the starting materials, were in general, known compounds; however, some of the alkyl morpholines employed have been synthesized for the first time according to the method described in the experimental part. For this purpose, the selected ethylene oxide derivative was acted upon by ethanolamine or some of its derivatives, and the formed amino-diol was cyclized with the elimination of water:

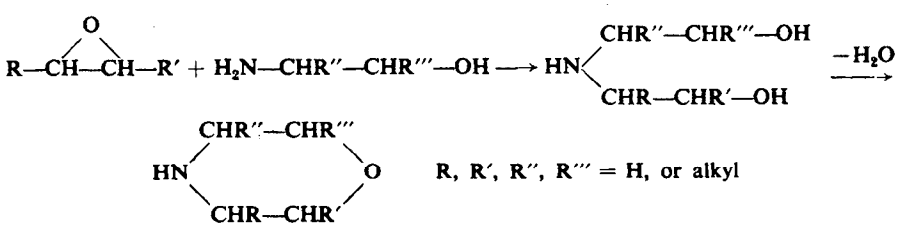
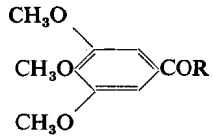


TABLE 1. AMIDES OF THE 3:4:5-TRIMETHOXYBENZOIC ACID



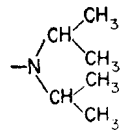
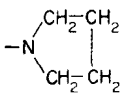
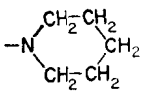
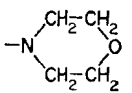
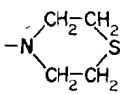
Compd.	R	Recryst. solvent	m.p., °C b.p., °C*	Empirical formula	Mol. wt.	Analysis % Calcd. C	H	N	OCH_3	Found C	H	N	OCH_3
1			100–104° a	$C_{16}H_{25}NO_4$	295·2			4·73	31·4			4·98	31·1
2			183–6°/ 0·3 mm* oil b	$C_{14}H_{19}NO_4$	265·2			5·28	35·1			5·50	34·9
3			206°/ 1 mm* oil c	$C_{15}H_{21}NO_4$	279·2			5·02	33·4			4·89	33·1
4		90% C_2H_5OH or H_2O	120–121°	$C_{14}H_{19}NO_5$	281·3	59·76	6·81	4·98	33·1	59·61	7·04	5·12	33·1
5		C_2H_5OH + H_2O (1:1)	102–103°	$C_{14}H_{19}O_4NS$	297·3	56·54	6·44	4·71	31·3	56·79	6·61	4·67	30·9

TABLE 1—*continued.*

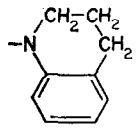
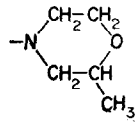
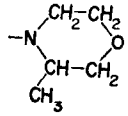
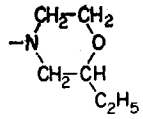
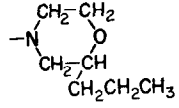
Compd.	R	Recryst. solvent	m.p., °C b.p., °C*	Empirical formula	Mol. wt.	Analysis (%) Calcd. C	H	N	OCH_3	Found C	H	N	OCH_3
6		petr. ether + $(CH_3)_2CO$ (2:1)	105–107°	$C_{19}H_{21}NO_4$	327·2	69·6	6·43	4·28	28·5	69·68	6·61	4·51	28·1
7		C_2H_5OH + H_2O (1:2·7)	114–117°	$C_{15}H_{21}NO_5$	295·2	61·0	7·12	4·74	31·6	61·3	7·18	4·79	31·7
8		C_6H_6 + petr. ether	94–98°	$C_{15}H_{21}NO_5$	295·2			4·74	31·6			4·87	31·2
9			190–192° 0·4 mm* oil	$C_{16}H_{23}NO_5$	309·2			4·53	30·1			4·75	29·8
10			208–211°/ 0·5 mm* oil	$C_{17}H_{25}NO_5$	323·2			4·33	28·8			4·54	29·1

TABLE 1—*continued.*

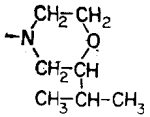
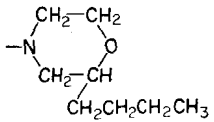
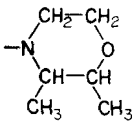
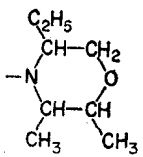
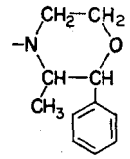
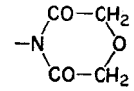
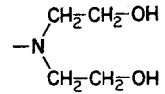
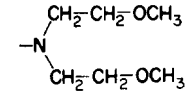
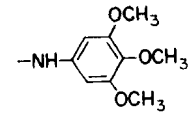
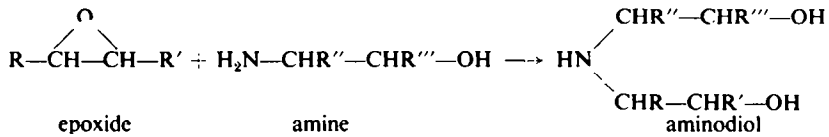
Compd.	R	Recryst. solvent	m.p., °C b.p., °C*	Empirical formula	Mol. wt.	Analysis % Calcd. C	H	N	OCH_3	Found C	H	N	OCH_3
11			196–200° 0·4 mm* oil	$C_{17}H_{25}NO_5$	323·2			4·33	28·8			4·51	29·1
12			39–40° 213–217°/ 0·6 mm*	$C_{18}H_{27}NO_5$	337·2	64·2	8·07	4·16		64·07	8·27	4·39	
13			164–165°/ 0·03 mm* oil	$C_{16}H_{23}NO_5$	304·3			4·53	30·1			4·78	30·58
14			157–163°/ 0·015 mm* oil	$C_{18}H_{27}NO_5$	337·4			4·15	27·4			4·53	27·2

TABLE 1—*continued.*

Compd.	R	Recryst. solvent	m.p., °C b.p., °C*	Empirical formula	Mol. wt.	Analysis % Calcd. C	H	N	OCH_3	Found C	H	N	OCH_3
15			Oil	$C_{21}H_{25}NO_5$	371·2			3·77	25·05			4·05	25·55
16		C_6H_6	138–140°	$C_{14}H_{15}NO_7$	309·1	54·40	4·86	4·53	30·1	54·54	4·99	4·40	30·7
17		CH_3OH + H_2O 5:1	131–133°	$C_{14}H_{21}NO_6$	299·3	56·2	7·07	4·68	31·2	56·35	7·30	4·67	30·8
18			199–202°/ 0·6 mm*	$C_{16}H_{25}NO_6$	327·3	58·70	7·67	4·28	47·4	58·76	7·68	4·28	46·8
19		C_2H_5OH	213–214°	$C_{19}H_{23}NO_7$	377·2	60·47	6·14	3·71	49·3	60·58	6·39	3·83	48·8

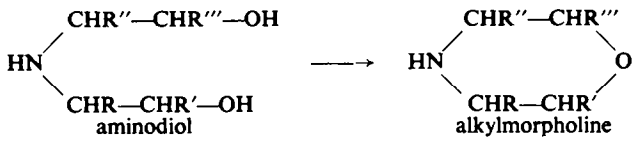
a, HCl salt, m.p. 141–143°. b, HCl salt, m.p. 158–160°. c, HCl salt, m.p. 133–134°.

TABLE 2. AMINODIOLS



Compd.	Aminodiol	Starting materials		B.p. °C	Empirical formula	Mol. wt.	N calcd.	N found
		Epoxide	Amine					
1	1-(2-hydroxyethyl)-amino-2-hydroxy-*n*-pentane, R=R″=R‴=H, R′=*n*-C_3H_7	1:2-epoxy-*n*-pentane, R=H, R′=*n*-C_3H_7	ethanolamine, R″=R‴=H	122–127° 1 mm	$C_7H_{17}NO_2$	147·1	9·52	9·34
2	1-(2-hydroxyethyl)-amino-2-hydroxy-3-methylbutane, R=R″=R‴=H, R′=*iso*-C_3H_7	1:2-epoxy-3-methylbutane R=H, R′=*iso*-C_3H_7	ethanolamine, R″=R‴=H	148° 12 mm	$C_7H_{17}NO_2$	147·1	9·52	9·73
3	1-(2-hydroxyethyl)-amino-2-hydroxyhexane, R=R″=R‴=H, R′=*n*-C_4H_9	1:2-epoxy-*n*-hexane, R=H, R′=*n*-C_4H_9	ethanolamine, R″=R‴=H	144° 5 mm m.p. 40–42°	$C_8H_{19}NO_2$	161·2	8·68	8·48
4	2-(hydroxyethyl)-amino-3-hydroxybutane R=R′=CH_3, R″=R‴=H	2:3-epoxybutane, R=R′=CH_3	ethanolamine, R″=R‴=H	107–109° 1 mm	$C_6H_{15}NO_2$	133·2	10·5	10·75
5	2-[(1-ethyl-2-hydroxy)-ethyl]-amino-3-hydroxybutane, R=R′=CH_3, R″=C_2H_5, R‴=H	2:3-epoxybutane, R=R′=CH_3	1-hydroxy-2-aminobutane, R″=C_2H_5, R‴=H	107° 0·8 mm	$C_8H_{19}NO_2$	161·2	8·68	8·75

TABLE 3. ALKYLMORPHOLINES



Compd.	Alkylmorpholine	B.p. °C	Empirical formula	Mol. wt.	N calcd.	N found
1	2-*n*-propylmorpholine, R=R″=R‴=H, R′=*n*-C_3H_7	150–153° 755 mm*	$C_7H_{15}NO$	129·1	10·85	11·03
2	2-*iso*-propylmorpholine, R=R″=R‴=H, R′=*iso*-C_3H_7	147–149° 750 mm	$C_7H_{15}NO$	129·1	10·85	10·69
3	2-*n*-butylmorpholine, R=R″=R‴=H, R′=*n*-C_4H_9	175–177° 763 mm†	$C_8H_{17}NO$	143·1	9·79	9·95
4	2:3-dimethylmorpholine, R=R′=CH_3, R″=R‴=H	145–148° 760 mm	$C_6H_{13}NO$	115·2	12·13	12·0
5	2:3-dimethyl-5-ethylmorpholine, R=R′=CH_3, R″=C_2H_5, R‴=H	160–165° 760 mm	$C_8H_{17}NO$	143·2	9·76	9·56

* N-tosyl derivative, m.p. 103–104°.
† N-tosyl derivative, m.p. 73–74°.

EXPERIMENTAL

Preparation of aminodiols

A solution of the epoxide (1 mole) in 96% ethanol (100 ml) was added dropwise into a stirred mixture of ethanolamine (or substituted ethanolamine) (5 moles) and 95% ethanol (270 ml) which was cooled in ice-water to maintain the temperature of the solution constantly below 4°. The addition required about one hour. After standing overnight at room temperature (in the case of 1-hydroxy-2-aminobutane a 24-hr boiling was necessary) the ethanol and excess ethanolamine was evaporated under reduced pressure. Fractionation of the residue *in vacuo* yielded the aminodiol. (See Table 2 for compounds produced.)

Preparation of alkylmorpholines

The aminodiol was added gradually under shaking to concentrated sulphuric acid (1 vol.) cooled by ice-water. The mixture was heated in a flask equipped with a condenser to 165° and then it was refluxed 8–10 hr in a bath of 165–180°. After cooling, the mixture was cautiously made alkaline and evaporated to dryness *in vacuo* on a bath of 85°. The residue was taken up in water (300 ml) and again evaporated to dryness and the process repeated in order to remove completely the alkylmorpholine by steam distillation. The distillate was acidified with hydrochloric acid, concentrated, the residue made alkaline and extracted with three 300 ml portions of ether. The solvent

was evaporated and the residue fractionated at atmospheric pressure to yield the alkylmorpholine. (See Table 3 for compounds produced.)

Preparation of the acid amides

The acid chloride (1 mole) was dissolved in anhydrous benzene (5 vols) and the corresponding amine (1·02 moles): furthermore triethylamine (1·05 moles) were added dropwise with stirring and cooling in ice-water. The reaction mixture was allowed to stand 1 hr, then it was refluxed for 2 hr. After cooling dilute hydrochloric acid was added, the benzene layer washed with dilute sodium bicarbonate solution and with water, dried over anhydrous potassium carbonate, concentrated under reduced pressure, and the residue was crystallized or distilled.

A few acid amides were prepared in a different way. These are described below.

N-(3:4:5-*Trimethoxybenzoyl*)-*diethanolamine*. A solution of 3:4:5-trimethoxybenzoyl chloride (34·7 g) in dry chloroform (700 ml) was added to a solution of diethanolamine (31·6 g) in dry chloroform (700 ml) with stirring and cooling in ice-water. After completed addition, the stirring was continued 1 hr more, then the reaction mixture was allowed to stand overnight. The lower layer was carefully separated, the chloroform distilled off under reduced pressure, and the remaining solid (47 g) repeatedly recrystallized from two 70 ml portions of methanol. Yield 32·2 g.

N-(3:4:5-*Trimethoxybenzoyl*)-*diethanolamine dimethyl ether*. Small portions of silver oxide (28 g) were added with shaking and cooling in ice-water into a mixture of N-(3:4:5-trimethoxybenzoyl)-diethanolamine (6·9 g), dry acetonitrile (70 ml) and methyl iodide (28 ml). After completed addition, the reaction mixture was shaken mechanically for 28 hr. The precipitate was filtered, washed with methanol, the solvent was evaporated from the filtrate *in vacuo*, and the remaining liquid (7·55 g) was fractionated under reduced pressure. B.p. 199–202° at 0·6 mm. Yield: 6·95 g.

PHARMACOLOGY

Methods

Toxicity

The toxicity of the compounds was determined on albino mice, weighing from 16 to 24 g, by intraperitoneal administration. The compounds were injected in four or five doses using suspensions prepared with Tween 80, to groups of ten mice. The results were recorded after 24 hr, and the LD_{50}'s calculated by Litchfield and Wilcoxon's probit method.[2]

Neurosedative action

The relative tranquillizing activity of the compounds was tested on mice by the method of Borsy *et al.*[3] Groups consisting of five mice were injected intraperitoneally and put after 1 hr into a Dew's photocell activity measuring chamber, where the orientational reflex of the animals was determined during a period of 30 min. The 50 per cent inhibition values were calculated from the inhibition percentages with reference to the activity of the control animals.

Analgesic action

This was tested on mice according to the hot plate method, modified by Pórszász and Herr.[4] The compounds under investigation were injected intraperitoneally then the pain reaction time of the animals was determined at 15 min intervals for 1 hr. Animals showing 2·5 times longer reaction time than normally, were taken as "positive". Based on the percentage of positive cases, the 50 per cent analgesic dose of the compounds were determined again by the probit method.[2]

Results

The pharmacological results obtained are shown in Table 4. The neuroplegic actions of the morpholid derivative had been reported by Borsy *et al.*[5] previously. It was found that the drug exhibited neuroplegic activity and low toxicity and exerted its depressive action without any muscle relaxing effect and with low toxicity. Its activity, when administered intraperitoneally on mice, was shown to be equivalent to that of Meprobamate, *per os* it was more effective. However, in contrast to Meprobamate, the compound inhibited the stimulating action of mescaline, morphine and benzpropamine. A narcosis potentiating activity and analgesic action was observed on mice and rats. In this latter respect, the compound was more effective than Carisoprodol. The analgesic action of this latter drug could not be demonstrated by the technique employed by us, and other methods[6] are to be used for the purpose.

According to our results, trimethoxybenzamide also has a neurosedative action. However, it is considerably more toxic than the parent compound. The same holds true for other heterocyclic amides (pyrrolidine, piperidine and thiomorpholine derivatives). Among the substituted morpholine derivatives, favourable properties were observed from the amide formed with 2-methylmorpholine. Although its inhibition of the orientation reflex of the animals equalled that of the morpholine derivative, its analgetic action was nearly twice as high. As an analgesic, the compound had only one-fifth of the activity of Dolantine (ethyl methylphenylpiperidine carboxylate). However, the action on mice was different from that of either synthetic analgesics or opiate alkaloids, since these drugs are known to act as stimulants, whereas the new compound had depressant properties.

Cleavage of the morpholine ring, i.e. formation of a diethanolamine derivative, suspended sedative effects, however, building up ether linkages on the hydroxyl groups restored the complete tranquillizing action. This fact clearly indicates the pharmacological importance of the internal ether linkage present in the morpholine ring.

Substitution of trimethoxybenzamide by open-chain alkyl groups results in the appearance of stimulant activity; thus, for example, diethylamide has stimulating action.[7]

The di*iso*propylamine derivative is considerably more toxic than the heterocyclic amines described by us, and excitant properties can be observed again instead of neuroplegic action.

Consequently, the compounds described are pharmacologically characterized by a depressant and simultaneous analgesic action, which however, effects do not necessarily parallel one another. The analgesic action may increase without any change in the tranquillizing effect, e.g. in the case of the 2-methylmorpholine derivative, and neuroplegic activity may be present without any analgetic action, as it appears in fact, e.g. in the diethanolamine dimethyl ether derivative.

Table 4. The toxicity, neurosedative and analgesic action of 3:4:5-trimethoxybenzamide derivatives in mice

Compd.	LD_{50} (mg/kg, i.p.)	ED_{50} (50% neurosedative dose) (mg/kg, i.p.)	AD_{50} (50% analgetic dose) (mg/kg, i.p.)
N-(3:4:5-trimethoxybenzoyl)-morpholine* (4)	1320·0	88·0	135·0
N-(3:4:5-trimethoxybenzoyl)-pyrrolidine (2)	650·0	90·0	120·0
N-(3:4:5-trimethoxybenzoyl)-piperidine (3)	525·0	205·0	104·0
N-(3:4:5-trimethoxybenzoyl)-thiomorpholine (5)	660·0	135·0	200·0
N-(3:4:5-trimethoxybenzoyl)-1:2:3:4-tetrahydroquinoline (6)	1100·0	180·0	200·0
N-(3:4:5-trimethoxybenzoyl)-2-methylmorpholine (7)	1080·0	82·0	64·0
N-(3:4:5-trimethoxybenzoyl)-3-methylmorpholine (8)	800·0	130·0	117·0
N-(3:4:5-trimethoxybenzoyl)-2-ethylmorpholine (9)	890·0	128·0	132·0
N-(3:4:5-trimethoxybenzoyl)-2-*n*-propylmorpholine (10)	980·0	110·0	112·0
N-(3:4:5-trimethoxybenzoyl)-2-*n*-butylmorpholine (12)	460·0	175·0	120·0
N-(3:4:5-trimethoxybenzoyl)-2:3-dimethylmorpholine (13)	500·0	200·0	145·0
N-(3:4:5-trimethoxybenzoyl)-2:3-dimethyl-5-ethylmorpholine (14)	1250·0	200·0	200 <
N-(3:4:5-trimethoxybenzoyl)-3:5-dioxomorpholine (16)	1250·0	400·0	200·0 <
N-(3:4:5-trimethoxybenzoyl)-diethanolamine (17)	2000·0	600·0 <	200·0 <
N-(3:4:5-trimethoxybenzoyl)-diethanolamine-dimethyl ether (18)	1500·0	95·0	200·0 <
3:4:5-trimethoxybenzamide	750·0	110·0	—
N-(3:4:5-trimethoxybenzoyl)-di*iso*propylamine (1)	45·0	It has a stimulant effect in 10–20 mg/kg	—
Meprobamate	680·0	92·0	150·0 <
Carisoprodol	980·0	150·0	150·0 <

* This compound is designated by United Works of Pharmaceutical and Dietetic Products (Budapest) under the trade name Trioxazin.

The figures in brackets refer to the compounds numbered accordingly in Table 1. Owing to their spare solubility, compounds 11, 15 and 19 we were not able

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