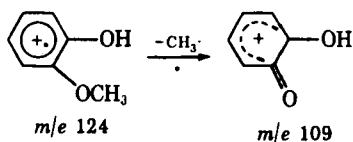
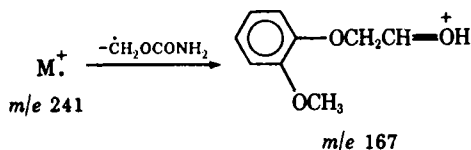


Scheme X—Fragmentations of mephenesin carbamate (R = CH₃) and methocarbamol (R = OCH₃)



Scheme XI



Scheme XII

of the expulsion of a methyl radical from the ion *m/e* 124 (Scheme XI).

The spectrum of methocarbamol (Fig. 2) also contains a weak, isolated fragment of *m/e* 167. An accurate mass measurement revealed an elemental composition of C₉H₁₁O₃, the formation of which is readily explained (Scheme XII).

The present study has shown that medicinal carbamates are readily characterized by means of their mass spectra.

REFERENCES

- (1) W. R. Benson and J. N. Damico, *J. Ass. Offic. Anal. Chem.*, **51**, 347(1968), and references contained therein.
- (2) S. W. Tam, *Org. Mass Spectrom.*, **2**, 729(1969).
- (3) B. Blessington, *ibid.*, **2**, 929(1969), and references contained therein.
- (4) W. H. Daly and C. W. Heurtevant, *Org. Mass Spectrom. (Suppl.)*, **4**, 165(1970).
- (5) W. Pereira, B. Halpern, M. D. Solomon, and A. M. Duffield, *Org. Mass Spectrom.*, **5**, 157(1971).
- (6) R. T. Coutts and R. A. Locock, *J. Pharm. Sci.*, **57**, 2096(1968).
- (7) R. A. Locock and R. T. Coutts, *Org. Mass Spectrom.*, **3**, 735(1970).
- (8) J. L. Shultz and A. G. Sharkey, *Anal. Chem.*, **28**, 926(1956).
- (9) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Mass Spectrometry of Organic Compounds," Holden-Day, San Francisco, Calif., 1967, p. 330.
- (10) F. W. McLafferty, *Anal. Chem.*, **28**, 306(1956).
- (11) E. L. Eliel, J. D. McCollum, S. Meyerson, and P. N. Rylander, *J. Amer. Chem. Soc.*, **83**, 2481(1961).
- (12) T. Aczel and H. E. Lumpkin, *Anal. Chem.*, **32**, 1819(1960).

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N-Trisubstituted Methylimidazoles as Antifungal Agents

S. CASADIO[▲], A. DONETTI, and G. COPPI

Abstract □ The preparation and the antifungal activity *in vitro* of nine *N*-trisubstituted methylimidazoles related to clotrimazole and of two congeneric compounds, having the 2-pyrrolylmethylidenimino and the 2-pyrimidinylamino groups in place of the imidazole nucleus, are presented. Significant activity against six different organisms was found for three compounds. The *in vivo* activity of one selected compound was determined against *Candida albicans*, *Aspergillus fumigatus*, and *Cryptococcus neoformans*.

Clotrimazole [1-(*o*-chloro- α,α -diphenylbenzyl)imidazole] (1) is a new type of antifungal, which has been reported (2) to possess broad spectrum activity in-

cluding that of amphotericin B and griseofulvin. The consistent results obtained in patients with severe systemic mycoses and the advantages that this drug

Keyphrases □ *N*-Trisubstituted methylimidazoles—synthesis, screened for antifungal activity □ Antifungal agents, potential—*N*-trisubstituted methylimidazoles synthesized and screened □ structure-activity relationships—*N*-trisubstituted methylimidazoles, antifungal activity

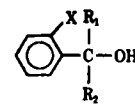


Table I—Trisubstituted Carbinols (I)

Number	R ₁	R ₂	X	Method	Crystallization Solvent ^a	Melting Point	Yield, % ^b	Molecular Formula	Analysis, %	
									Calc.	Found
1	H		Cl	A	C-P	87–88°	65	C ₁₇ H ₁₃ ClO	C 75.98 H 4.87 Cl 13.19	75.65 4.62 13.52
2			Cl	B	P	152–154°	55	C ₂₂ H ₁₇ ClO	C 80.11 H 4.97 Cl 10.28	79.73 4.82 10.12
3			Cl	A ^c	—	— ^d	41	C ₂₂ H ₁₇ ClO	C 80.11 H 4.97 Cl 10.28	80.22 4.58 10.45
4			Cl	B	P	147–148°	82	C ₂₄ H ₁₉ ClO	C 80.96 H 5.16 Cl 9.56	80.77 5.25 9.66
5			Cl	A ^c	C-P	143–144°	62	C ₃₁ H ₂₃ ClO	C 83.30 H 5.19 Cl 7.93	82.51 5.16 7.97
6			Cl	B	E-H ₂ O	125–126°	38	C ₁₀ H ₁₁ ClNO	C 73.09 H 4.77 Cl 11.98 N 4.73	73.15 4.90 11.71 4.76
7			H	A	Et-P	114–115° ^e	62	C ₁₀ H ₁₁ NOS	C 71.88 H 4.90 N 5.24 S 11.99	71.71 4.85 5.36 11.78
8			Cl	A	E	137–138° ^f	50	C ₁₉ H ₁₃ ClO	C 77.95 H 4.47 Cl 12.11	77.72 4.68 12.38
9			H	B	C	131–132°	86	C ₁₉ H ₁₃ ClO	C 77.95 H 4.47 Cl 12.11	78.15 4.35 12.31

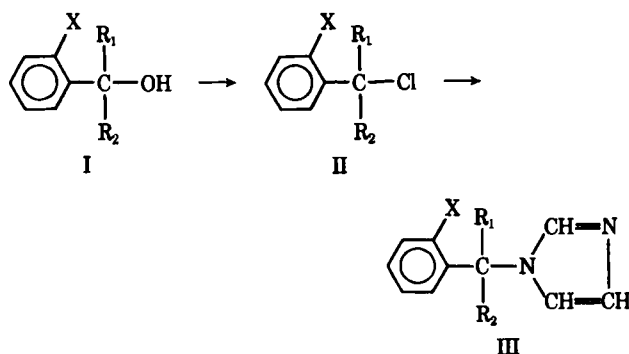
^a B = benzene, C = cyclohexane, E = ethanol, Et = ether, and P = petroleum ether. ^b Crystallized product. ^c Chromatographed on a silica gel column using benzene-carbon tetrachloride (2:8) as the eluent. ^d Glassy solid. ^e R. P. Kurkij and E. V. Brown, *J. Amer. Chem. Soc.*, **74**, 6260(1952), reported m.p. 116–117°. ^f N. Campbell and A. Marks, *J. Chem. Soc.*, 1951, 2941, reported m.p. 140–141°.

appears to possess over the classical antifungal agents (3) encouraged the synthesis and the examination of some congeners.

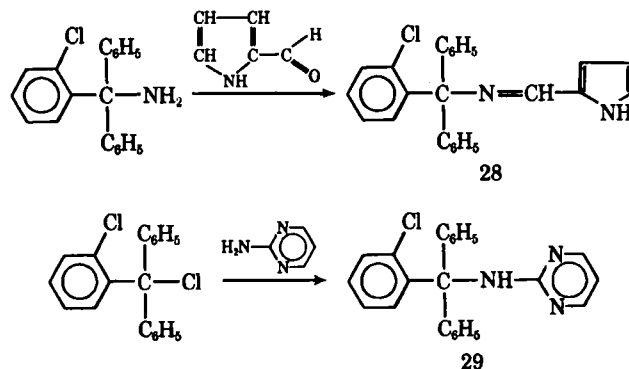
The synthesis of the title compounds (Scheme I) was achieved starting from the corresponding carbinols (I, Table I), obtained by reaction of the required lithium or Grignard reagent with the appropriate carbonyl compound. Treatment of the carbinols with thionyl chloride afforded the corresponding chlorides (II, Table II) which

were, in turn, converted into *N*-substituted imidazoles (III, Table III) by reaction with imidazole.

Compounds 28 and 29 (Table IV), in which the imidazole moiety of clotrimazole was replaced by the 2-pyrrolylmethylideneimino and 2-pyrimidinylamino groups, were prepared by condensing 2-pyrrolaldehyde and 2-aminopyrimidine with bisphenyl-*o*-chlorophenylcarbinylamine and bisphenyl-*o*-chlorophenylcarbinyl chloride, respectively (Scheme II).



Scheme I



Scheme II

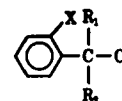


Table II—Trisubstituted Methyl Chlorides (II)

Number	R ₁	R ₂	X	Method	Crystal- lization Solvent ^a	Melting Point	Yield, %	Molecular Formula	Analysis, % Calc. Found	
10	H		Cl	A	P	117–118°	95	C ₁₇ H ₁₃ Cl ₂	Cl 24.69	24.35
11			Cl	B	—	—	—	C ₂₃ H ₁₆ Cl ₂	Cl 19.52	19.73
12			Cl	A	—	— ^c	90	C ₂₁ H ₁₆ Cl ₂	Cl 19.52	19.03
13			Cl	A	Et	168–169°	84	C ₂₅ H ₁₈ Cl ₂	Cl 18.21	18.53
14			Cl	A	P	160–161°	88	C ₃₁ H ₂₂ Cl ₂	Cl 15.23	15.51
15			Cl	B	P	137–138°	94	C ₁₈ H ₁₃ Cl ₂ N	Cl 22.56 N 4.46	22.31 4.35
16			H	A	—	— ^d	93	C ₁₆ H ₁₂ CINS	Cl 12.40 N 4.90	12.52 4.81
17			Cl	A	P	142–143°	90	C ₁₉ H ₁₃ Cl ₂	Cl 11.22	10.95
18			H	A	P	109–110°	89	C ₁₉ H ₁₃ Cl ₂	Cl 22.78	22.51

^a See Footnote a of Table I. ^b Glassy product. ^c Waxy product. ^d Thick oil.

The antifungal activity of the compounds was studied *in vitro* against six different organisms (Table V). After this preliminary screening, the *in vivo* activity of one selected compound was determined against *Candida albicans*, *Aspergillus fumigatus*, and *Cryptococcus neoformans* in mice infections (Table VI).

EXPERIMENTAL¹

The intermediate trisubstituted carbinols (I) were synthesized by the action of the required Grignard or lithium reagent on the appropriate carbonyl compound. The characteristics of these carbinols are listed in Table I, and their preparation is illustrated by the following methods.

Bis(4-biphenyl)-*o*-chlorophenylcarbinol (Compound 5, Table I)—*Method A*—A solution of 13.36 g. (0.04 mole) of 4,4'-diphenylbenzophenone (4) in 150 ml. of dry benzene was added dropwise to a Grignard reagent prepared from 23 g. of *o*-chlorobromobenzene (0.12 mole) and 2.52 g. of magnesium turnings (0.104 mole) in 75 ml. of dry ether. The mixture was stirred for 1 hr. at room temperature and then refluxed for an additional 1 hr. The reaction mixture was decomposed with 10% sulfuric acid, and the organic layer was washed with water, saturated sodium carbonate solution, and water. Removal of the solvent left a thick oil, which was steam distilled until no appreciable amount of water-insoluble material was present

in the distillate (2 hr.). The undistilled carbinol was extracted with ether, and the extract was dried over anhydrous magnesium sulfate. After evaporation of the solvent, the residue was chromatographed on silica gel, using benzene-carbon tetrachloride (2:8) as the eluent, and then crystallized from cyclohexane-petroleum ether to give 11.07 g. of Compound 5 as colorless crystals.

The following carbinols (I) were prepared in an analogous manner: Compound 1 (from *o*-chlorobenzaldehyde and 1-naphthylmagnesium bromide), Compound 3 [from 2-benzoylnaphthalene (5) and *o*-chlorophenylmagnesium bromide], Compound 7 [from benzophenone and 2-thiazolylmagnesium bromide (6)], and Compound 8 (from fluorenone and *o*-chlorophenylmagnesium bromide).

9-Phenyl-1-chlorofluoren-9-ol (Compound 9, Table I)—*Method B*—A solution of 7.34% phenyllithium in 280 ml. of ether was added to a stirred solution of 35 g. 1-chlorofluorenone (0.163 mole) (7) in 600 ml. of dry ether. After 19 hr. of stirring at room temperature, the mixture was decomposed with water and extracted with ether. The ethereal layer was washed with water until neutral, dried over anhydrous magnesium sulfate, and evaporated to dryness to give 45.6 g. of a light-brown waxy product which, on crystallization, furnished 41.04 g. of Compound 9 as colorless crystals.

Compound 2 [from *o*-chlorobenzophenone (8) and 1-naphthyllithium], Compound 4 [from 4-(2-chlorobenzoyl)-biphenyl (9) and phenyllithium], and Compound 6 (from *o*-chlorobenzophenone and 2-pyridyllithium) were prepared by a similar procedure.

Trisubstituted Methyl Chlorides (II, Table II)—*Method A*—A solution of 0.1 mole of the appropriate carbinol in 150 ml. of thionyl chloride was refluxed for 4 hr. The excess thionyl chloride was evaporated under reduced pressure, and the residue was used as crude product or crystallized.

Method B—A solution of 0.04 mole of thionyl chloride in 10 ml. of dry benzene was added dropwise to a stirred solution of 0.02 mole of the carbinol and 0.03 mole of pyridine in 80 ml. of benzene. The mixture was refluxed for 5 hr., cooled, and then poured into excess

¹ All melting points were determined on a Büchi capillary melting-point apparatus and are uncorrected. The IR spectra were determined on a Perkin-Elmer 337 spectrophotometer; the NMR spectra were taken using a Varian A 60-A instrument and tetramethylsilane as the internal standard. IR and NMR spectra agreed in each case with the structural assignments.

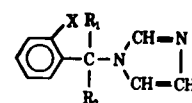


Table III—*N*-Trisubstituted Methylimidazoles (III)

Number	R ₁	R ₂	X	Crystallization Solvent ^a	Melting Point	Yield, % ^b	Time, hr.	Molecular Formula	Analysis, % Calc. Found	
19	H		Cl	P	145–146°	38	5	C ₂₀ H ₁₅ ClN ₂	C 75.35 H 4.74 Cl 11.12 N 8.79	75.12 4.52 11.31 8.51
20			Cl	— ^c	120–121°	51	30	C ₂₆ H ₁₉ ClN ₂	C 79.08 H 4.85 Cl 8.98 N 7.09	78.88 4.91 8.53 6.61
21			Cl	Et	197–199°	48	42	C ₂₆ H ₁₉ ClN ₂	C 79.08 H 4.85 Cl 8.98 N 7.09	78.91 4.68 8.58 6.95
22			Cl	Et	169–170°	78	44	C ₂₈ H ₂₁ ClN ₂	C 79.89 H 5.03 Cl 8.42 N 6.65	79.57 4.82 8.30 6.43
23			Cl	Et	206–207°	78	40	C ₃₄ H ₂₅ ClN ₂	C 82.16 H 5.07 Cl 7.13 N 5.63	82.33 4.95 6.92 5.55
24			Cl	Et	150–151°	68	40	C ₂₁ H ₁₆ ClN ₂	C 72.93 H 4.66 Cl 10.25 N 12.15	72.75 4.49 10.12 12.31
25			H	Et	212–214°	86	20	C ₁₉ H ₁₅ N ₂ S	C 71.90 H 4.76 N 13.24 S 10.10	72.10 4.90 13.50 9.75
26			Cl	P	160–161°	84	20	C ₂₂ H ₁₆ ClN ₂	C 77.08 H 4.41 Cl 10.34 N 8.17	76.95 4.33 10.12 8.31
27			H	P	135–136°	62	40	C ₂₂ H ₁₅ ClN ₂	C 77.08 H 4.41 Cl 10.34 N 8.17	76.85 4.36 9.98 8.25

^a See Footnote a of Table I. ^b Crystallized product. ^c Purified by column chromatography on silica gel using benzene–ether (1:1) as the eluent.

water. The organic layer was washed with water, dried over anhydrous magnesium sulfate, and evaporated to dryness. The products were isolated as shown in Table II.

***N*-Trisubstituted Methylimidazoles (III, Table III)**—A solution of 0.1 mole of the appropriate chloride and 0.3 mole of imidazole was refluxed in 120 ml. of dry benzene for the time indicated in Table III. After cooling to room temperature, the mixture was poured into water and extracted with benzene. The organic layer was washed with water, dried over anhydrous magnesium sulfate, and evaporated to dryness; the residue was purified as shown in Table III.

Bisphenyl-*o*-chlorophenylcarbonylamine—A solution of 31.32 g. (0.1 mole) of bisphenyl-*o*-chlorophenylcarbonyl chloride (1) in 900 ml. of ether was added under stirring to 900 ml. of 32% ammonia solution. After 22 hr. of stirring at room temperature, the organic layer was separated, extracted with 10% hydrochloric acid, and washed with water. The acidic solution was combined with the aqueous washings and filtered with charcoal, and the filtrate was basified with 10% sodium hydroxide. The basic material was extracted with ether, and the extract was washed with water, dried over anhydrous magnesium sulfate, and evaporated to dryness to give 24 g. of a white solid (81.7% yield, m.p. 95–97°).

***N*-Bisphenyl-*o*-chlorophenyl-2-pyrrylmethylideniminomethane (Compound 28, Table IV)**—A solution of 8.81 g. (0.03 mole) of bis-

phenyl-*o*-chlorophenylcarbonylamine and 7.13 g. (0.075 mole) of 2-pyrrolaldehyde (10) in 50 ml. of dry benzene was refluxed in the presence of catalytic amounts of *p*-toluenesulfonic acid, while the water azeotrope was removed with a takeoff adapter. After all of the water had been removed (23 hr.), the solution was allowed to cool to room temperature; the product, which crystallized on standing, was collected by filtration.

Bisphenyl-*o*-chlorophenyl-2-pyrimidinylaminomethane (Compound 29, Table IV)—A solution of 6.26 g. (0.02 mole) of bisphenyl-*o*-chlorophenylmethyl chloride and 7.61 g. (0.08 mole) of 2-aminopyrimidine in dry benzene was refluxed for 43 hr. The solution was then cooled, diluted with benzene, washed with water, and dried over anhydrous magnesium sulfate. Removal of the solvent left a yellow, thick oil which was purified by crystallization.

Microbiological Experiments (11)—In Vitro—The test organisms used in this experiment were *C. albicans* (DM), *Crypt. neoformans* (ATCC 5006), *Nocardia asteroides* (C.B.S.), *Trichophyton mentagrophytes* (I.D.A.), *A. niger* (I.S.M.), and *Penicillium chrysogenum* (I.D.A.). The organisms were incubated for 48 hr. at 37° (*C. albicans*, *Crypt. neoformans*, and *N. asteroides*) and for 96 hr. at 25° (*T. mentagrophytes*, *A. niger*, and *P. chrysogenum*) in Sabouraud broth (Difco). The cultures were then centrifuged and washed twice with sterile 0.9% saline and were finally resuspended in sterile saline. They were then diluted with sterile saline to give 90% light trans-

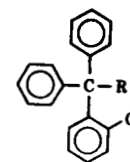


Table IV—Chlorotrityl Derivatives Bearing 2-Pyrrylmethylidenimino and 2-Pyrimidinylamino Groups (IV)

Number	R	Crystallization Solvent ^a	Melting Point	Yield, % ^b	Molecular Formula	Analysis, %	
						Calc.	Found
28		B	152–153°	57	C ₂₁ H ₁₉ ClN ₃	C 77.72 H 5.16 Cl 9.56 N 7.55	77.95 5.32 9.72 7.83
29		E	159–160°	61	C ₂₁ H ₁₈ ClN ₃	C 74.29 H 4.88 Cl 9.53 N 11.30	74.15 4.95 9.62 11.15

^a See Footnote a of Table I. ^b Crystallized product.

Table V—*In Vitro* Antifungal Activity of *N*-Trisubstituted Methylimidazoles^a

Test Organism	Compound												Clo- trima- zole	Nys- tatin
	19	20	21	22	23	24	25	26	27	28	29			
<i>C. albicans</i>	>100	100	>100	>100	>100	50	>100	50	3.12	>100	>100	3.12	0.40	
<i>Crypt. neoformans</i>	>100	>100	>100	>100	>100	0.80	>100	0.10	6.25	>100	>100	0.40	0.40	
<i>N. asteroides</i>	>100	0.40	6.25	6.25	>100	3.12	100	0.10	3.12	50	12.5	1.56	50	
<i>T. mentagrophytes</i>	0.20	0.40	>100	>100	>100	1.56	0.80	0.10	0.40	>100	>100	0.10	0.20	
<i>A. niger</i>	>100	>100	>100	>100	>100	6.25	>100	0.80	3.12	>100	>100	1.56	3.12	
<i>P. chrysogenum</i>	3.12	>100	>100	>100	>100	0.40	>100	0.10	1.56	>100	>100	0.80	1.56	

^a Minimum inhibitory concentrations in micrograms per milliliter.

Table VI—*In Vivo* Antifungal Activity

Compound Tested	<i>C. albicans</i>		<i>A. fumigatus</i>		<i>Crypt. neoformans</i>	
	Number Animals Dead/Number Treated	Efficacy, %	Number Animals Dead/Number Treated	Efficacy, %	Number Animals Dead/Number Treated	Efficacy, %
27	26/48	45.84	23/29	20.69	27/27	0.00
Clotrimazole	23/49	53.07	20/30	33.34	27/27	0.00
Nystatin	30/65	53.85	15/29	48.28	23/65	64.62

mission at 625-nm. wavelength on the colorimeter².

In all cases, exactly 0.05 ml. of this standardized culture was used for the inoculum. Compounds to be tested were suspended with 0.1% of polysorbate 80³ in sterile distilled water and serially diluted in Sabouraud broth. The inoculated tubes were incubated as already described and were finally observed for growth. Nystatin and clotrimazole were used as reference standards.

In Vivo—The test organisms in this study were *C. albicans* (DM), *Crypt. neoformans* (ATCC 5006), and *A. fumigatus* (I.I.). The organisms were incubated for 24 hr. at 37° (*C. albicans* and *Crypt. neoformans*) in Gremy broth and for 96 hr. at 24° (*A. fumigatus*) in Sabouraud broth. The cultures were then centrifuged and washed twice with sterile 0.9% saline and were finally resuspended in sterile saline to give an appropriate light transmission at 625 nm. (*C. albicans*, *T* = 15%; *Crypt. neoformans*, *T* = 20%; and *A. fumigatus*, *T* = 2%). The inoculum used for each mouse was 0.15 ml. of these suspensions intravenously. All infected and untreated animals were dead within 9 days. Compound 27 and clotrimazole were administered orally and nystatin was administered subcutaneously suspended in 5% acacia gum (10 ml./kg.) at the dose of 100 mg./kg. The administrations were performed on the 1st day 2 hr. before and 3 and 6 hr. after infections and on the 2nd, 3rd, and 4th days at 9 a.m. and 4 p.m. All mice were observed for a period of 9 days.

² Spectronic 20, Bausch & Lomb, Rochester, N. Y.

³ Tween 80.

RESULTS AND DISCUSSION

Compounds 19–29 were subjected to preliminary antifungal screening. The *in vitro* activity against six different organisms is reported in Table V. On the basis of this preliminary screening, it would appear that the ring size of the substituents plays some part in antifungal activity of compounds of the clotrimazole type. In fact, replacement of phenyl groups in the clotrimazole molecule by α - or β -naphthyl groups (Compounds 19, 20, and 21) or substitution in the *para* position by phenyl radicals (Compounds 22 and 23) led to compounds practically devoid of antifungal activity. No inhibition was also observed for the thiazole Compound 25 and when the imidazole was replaced by different groups (Compounds 28 and 29). The activity was retained when one of the two phenyl groups of clotrimazole was replaced by a 2-pyridyl radical (Compound 24) and in Compounds 26 and 27, where two phenyl groups are *ortho* connected to form a fluorene system. Actually, Compounds 24 and 26 inhibited *in vitro* the growth of the considered organisms, with the exception of *C. albicans*. In this respect, the activity of Compound 26 was even better than that of clotrimazole. On the other hand, Compound 27 was less active than Compound 26, but it showed inhibition against *C. albicans*. The activity of the latter compound was only slightly inferior to that of the reference standards.

During the completion of this study, the authors became acquainted with two patents (12, 13) reporting the preparation and antifungal activity of compounds having formulas corresponding to Compounds 24 and 26, so only Compound 27 was chosen for the study of the *in vivo* activity (Table VI). Compound 27 confirmed its

activity *in vivo* against *C. albicans* and *A. fumigatus*. Like clotrimazole, it was inactive against *Crypt. neoformans*.

The data obtained in this study indicate that Compound 27 is endowed with good inhibitory activity *in vitro* and *in vivo*, which is only slightly inferior to that of clotrimazole.

REFERENCES

- (1) K. H. Büchel (to Farbenfabriken Bayer A.-G.), Netherlands pat. 6.813.715 (1968); for a recent reference see also K. H. Büchel, W. Draber, E. Regel, and M. Plempel, *Arzneim.-Forsch.*, **22**, 1260(1972).
- (2) M. Plempel, K. Bartmann, K. H. Büchel, and E. Regel, *Deut. Med. Wochenschr.*, **94**, 1356(1969).
- (3) H. Oberste-Lehn, I. Baggesen, and M. Plempel, *ibid.*, **94**, 1365(1969); for a recent reference see A. Freis, *Arzneim.-Forsch.*, **22**, 1289(1972), and following papers.
- (4) C. Musante and P. Giraldi, *Gazz. Chim. Ital.*, **78**, 662(1948).
- (5) A. Schönberg, *Chem. Ber.*, **58**, 580(1925).
- (6) R. P. Kurkijy and E. V. Brown, *J. Amer. Chem. Soc.*, **74**, 6260(1952).
- (7) N. Kharasch and T. C. Bruice, *ibid.*, **73**, 3240(1951).
- (8) E. Berliner, *ibid.*, **66**, 534(1944).

(9) N. P. Buu-Hoi, Nguyen-Hoan, J. Lecocq, and M. de Clercq, *Rec. Trav. Chim. Pays-Bas*, **67**, 795(1948).

(10) R. M. Silverstein, E. E. Ryskiewicz, and C. Willard, "Organic Syntheses," collect. vol. IV, Wiley, New York, N. Y., 1963, p. 831.

(11) G. Coppi, C. Ciani-Bonardi, and R. Genova, *Boll. Chim. Farm.*, **108**, 421(1969).

(12) W. Draber, K. H. Büchel, and M. Plempel (to Farbenfabriken Bayer A.-G.), S. African pat. 69.04,723 (1970); through *Chem. Abstr.*, **73**, 35370e(1970).

(13) W. Draber, K. H. Büchel, and M. Plempel (to Farbenfabriken Bayer A.-G.), Ger. pat. 1,811,654 (1970); through *Chem. Abstr.*, **73**, 45510q(1970).

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Toxicological and Pharmacological Actions of Methacrylate Monomers I: Effects on Isolated, Perfused Rabbit Heart

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Abstract □ A series of 12 methacrylate esters and methacrylic acid was investigated for activity upon the isolated, perfused rabbit heart. Each compound was dissolved in the perfusing fluid and tested at concentrations of 1:1000, 1:10,000, and 1:100,000 (v/v). All compounds reduced heart rate and force of contraction at one or more of these concentrations, and most, but not all, of the compounds reduced coronary flow rate.

Keyphrases □ Methacrylate monomers—toxicological and pharmacological actions, isolated, perfused rabbit heart □ Medical devices—toxicity and pharmacological actions of methacrylate esters from dental and surgical uses □ Methyl acrylic acid esters—toxicity and pharmacological actions, related to use in dentistry and surgery, cardiovascular effects, isolated, perfused rabbit heart □ Toxicity—methacrylate monomers, effects on isolated, perfused rabbit heart

Methacrylate esters are esters of methyl acrylic acid, $\text{CH}_2=\text{CH}(\text{CH}_3)\text{COOH}$, and the lower members of the series have a penetrating, disagreeable odor which may be recognized in tissues of treated animals (1). These esters may be polymerized by heat, oxygen, and oxygen-yielding compounds. The methacrylate polymers are widely used in dentistry and surgery as well as in many industrial applications.

Toxicity studies by Deichmann (1) and by Spealman *et al.* (2) indicated that lower members of the methacrylate esters were acutely lethal to laboratory animals due to respiratory depression by all routes of administration employed. Lawrence *et al.* (3) subjected 11 of the present 13 compounds to structure-toxicogenic analy-

ses and found eight of these 11 compounds to be consistent with the hypothesis of a common mode of lethal activity.

Various investigators have reported a mild to marked irritant effect from application of some methacrylate monomers to skin, rabbit eye, wall of the stomach, respiratory membranes, and peripheral nerve tissue (1, 2, 4, 5). Strain (6) reported contact allergic reactions in sensitive patients to the residual monomer from incompletely polymerized methyl methacrylate dentures. Chronic toxicity feeding studies were conducted on rats and dogs by Borzelleca *et al.* (7) over a 2-year period. They did not find an increased mortality in the methacrylate-treated animals and, in fact, noted only a few instances of abnormal findings.

Acrylic polymers, primarily self-curing methyl methacrylate, have found some use as "bone cements" in orthopedics, especially for intramedullary placement of prosthetic devices. Hypotensive responses in patients shortly after clinical use of the cement have been reported by several workers (8–10). Cardiac arrests, some of which were fatal, have been reported in a few patients undergoing such treatment (9, 10) in which methyl methacrylate monomer was suggested as the primary or contributory cause of the cardiac arrest (10).

The present study was undertaken to investigate the pharmacological and toxicological effects of methacrylate monomers upon cardiac function, independent of their effect upon respiration and peripheral vasodilator activity.