indicated, tests were performed at times of peak activity as determined during toxicity studies.

Determinations were made of 24-hr toxicities, using groups of ten mice at each dose level, of anticonvulsant activity against electroshock and pentylenetetrazole,[4] and of ability to protect against strychnine lethality[5] and amphetamine aggregation lethality,[6] the dose used in the latter test being the same as that in the strychnine test in nearly all cases. Representative compounds were also screened for analgetic[7] and antiemetic[8] activity.

Acknowledgment.—We are indebted to Dr. J. P. Heeschen, of the Chemical Physics Laboratory, The Dow Chemical Co., for determination and interpretation of nmr spectra.

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Hypotensive Quaternary Ammonium Salts with a Guaiacol or Thymol Residue

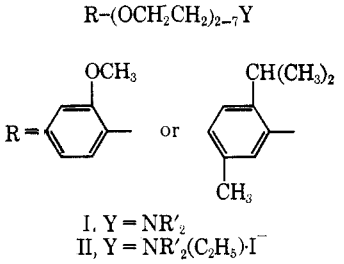
M. CARISSIMI, A. CATTANEO, R. D'AMBROSIO, E. GRUMELLI, E. MILLA, AND F. RAVENNA

Research Laboratories of Maggioni and Co., S.p.A., 20133 Milan, Italy

Received November 17, 1967

In a previous paper[1] we described a series of basic ethers of guaiacol and thymol with a polyoxyethylenic chain (I), some of which showed considerable antitussive activity; in addition, in almost all of the compounds of that series, we recorded hypotensive properties of short duration, probably originating in a direct action on the myocardium or the peripheral vasodilation. Quaternary ammonium salts often show a pronounced activity on neuromuscular or ganglionic transmission, which accounts for their properties of lowering blood pressure; this prompted us to transform the basic ethers previously described into quaternary ammonium salts (II), in order to see if the hypotensive activity of the former was enhanced.

The description of the new compounds, listed in Table I, and their pharmacological evaluation are the subject of the present note.



I, Y = NR'_2
II, Y = $NR'_2(C_2H_5)I^-$

Experimental Section

Where analyses are indicated only by symbols of the elements analytical results obtained for those elements were within ±0.4% of the theoretical values.

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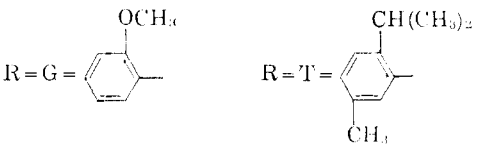
TABLE I

ETHIODIDES OF BASIC ETHERS OF GUAIACOL AND THYMOL

$R(OCH_2CH_2)_nNR'_2C_2H_5I^-$



No.	R	n	NR'_2	Formula[f]	Method	Yield, %
1	G	2	$N(C_2H_5)_2$	$C_{17}H_{30}INO_3$	A	67[a,b]
2	T	2	$N(C_2H_5)_2$	$C_{20}H_{36}INO_2$	A	73[c]
3	G	3	$N(C_2H_5)_2$	$C_{19}H_{34}INO_4$	B	75
4	T	3	$N(C_2H_5)_2$	$C_{22}H_{40}INO_3$	A	69[d]
5	G	4	$N(C_2H_5)_2$	$C_{21}H_{38}INO_5$	B	87
6	T	4	$N(C_2H_5)_2$	$C_{24}H_{44}INO_4$	B	69
7	G	5	$N(C_2H_5)_2$	$C_{23}H_{42}INO_6$	B	78
8	T	5	$N(C_2H_5)_2$	$C_{26}H_{48}INO_5$	B	79
9	G	6	$N(C_2H_5)_2$	$C_{25}H_{46}INO_7$	B	81
10	T	6	$N(C_2H_5)_2$	$C_{28}H_{52}INO_6$	B	80
11	G	7	$N(C_2H_5)_2$	$C_{27}H_{50}INO_8$	B	77
12	T	7	$N(C_2H_5)_2$	$C_{30}H_{56}INO_7$[e]	B	82
13	G	2	Piperidino	$C_{18}H_{30}INO_3$	B	81
14	T	2	Piperidino	$C_{21}H_{36}INO_2$	B	91
15	G	3	Piperidino	$C_{20}H_{34}INO_4$[f]	B	95
16	T	3	Piperidino	$C_{23}H_{40}INO_3$	B	92
17	G	4	Piperidino	$C_{22}H_{38}INO_5$	B	94
18	T	4	Piperidino	$C_{25}H_{44}INO_4$	B	89
19	G	5	Piperidino	$C_{24}H_{42}INO_6$	B	93
20	T	5	Piperidino	$C_{27}H_{48}INO_5$	B	90
21	G	6	Piperidino	$C_{26}H_{46}INO_7$	B	91
22	T	6	Piperidino	$C_{29}H_{52}INO_6$[g]	B	90
23	G	2	Morpholino	$C_{17}H_{28}INO_4$	B	56
24	T	2	Morpholino	$C_{20}H_{34}INO_4$[h]	B	32
25	G	5	Morpholino	$C_{19}H_{32}INO_6$	B	80
26	T	3	Morpholino	$C_{22}H_{38}INO_4$	B	73
27	G	1	Morpholino	$C_{21}H_{36}INO_6$	B	87
28	T	4	Morpholino	$C_{24}H_{42}INO_5$	B	79
29	G	2	Pyrrolidino	$C_{17}H_{28}INO_3$	B	96
30	T	2	Pyrrolidino	$C_{20}H_{34}INO_2$	B	95
31	G	3	Pyrrolidino	$C_{19}H_{32}INO_4$	B	72
32	T	3	Pyrrolidino	$C_{22}H_{38}INO_3$	B	87
33	G	4	Pyrrolidino	$C_{21}H_{36}INO_5$	B	95
34	T	4	Pyrrolidino	$C_{24}H_{42}INO_4$	B	86
35	G	2	1-Methylpiperazino	$C_{20}H_{36}I_2N_2O_3$	C	68
36	T	2	4-Methylpiperazino	$C_{23}H_{42}I_2N_2O_2$[i]	C	81[j]
37	G	3	4-Methylpiperazino	$C_{22}H_{40}I_2N_2O_4$	C	66
38	T	3	4-Methylpiperazino	$C_{25}H_{46}I_2N_2O_3$	C	60
39	G	1	4-Methylpiperazino	$C_{24}H_{44}I_2N_2O_5$[k]	C	86
40	T	4	4-Methylpiperazino	$C_{27}H_{50}I_2N_2O_4$	C	65

[a] Melting points were determined in a capillary tube and are not corrected. [b] Mp 94° from *i*-PrOH. [c] Mp 109° from *i*-PrOH–Et_2O. [d] Mp 66–68° (washed many times with ether). [e] I: calcd, 18.95; found, 18.46. [f] I: calcd, 26.47; found, 25.94. [g] I: calcd, 19.90; found, 20.35. [h] I: calcd, 27.38; found, 26.89. [i] Mp 163° from *i*-PrOH. [j] I: calcd, 40.12; found, 40.65. [k] I: calcd, 36.55; found, 37.17. [l] All compounds were analyzed for I, N.

Methods A and B.—The amine was dissolved with cooling in the same volume of EtI and, after standing 24 hr in the dark at room temperature, dry ether was added to the solution. Sometimes a solid precipitated (method A). This was filtered, washed with ether, and recrystallized. In most cases, however, an oil separated (method B) which was repeatedly slurried with ether and dissolved in 10 vol of acetone. After filtering with charcoal the solution was evaporated, yielding the quaternary salt as a clear water-soluble oil, which was dried at 60° (1 mm).

Method C.—The amine (5 mmoles), 5 ml of EtI, and 50 ml of dry MeOH were refluxed for 16 hr, after which time the solution was evaporated to dryness. The oily residue was slurried repeatedly with dry ether and dissolved in 20 ml of a saturated solution of $NaHCO_3$. This solution was extracted five times with 5 ml ($CHCl_3$) and evaporated at 35° (13 mm) to give a semisolid residue, from which the mineral salts were eliminated by extracting ing with 20-ml portions of hot *i*-PrOH and filtering from insoluble material. After evaporation of the solvent the oily quaternary salt was checked for the presence of mineral residue and extracted with *i*-PrOH until it was pure.

Pharmacology

All the compounds shown in Table I were studied as spasmolytics, as hypotensives, and, owing to the presence of a quaternary nitrogen, as possible synaptic blocking agents at ganglionic and neuromuscular level.

As spasmolytics they were tested against acetylcholine, $BaCl_2$, and nicotine contraction on the isolated jejunum of rats and guinea pigs.[2] Their effect on blood pressure and their possible synaptic blocking activity were tested on rabbits and cats anesthetized with pentobarbital, injected intravenously with 5.5 μM/kg of the various substances.

In cats (a) the two nictitating membranes were electrically stimulated at the same time, pre- and and postganglionically, respectively, before as well as during and after treatment and their isotonic contractions were kymographically recorded; (b) the changes, after treatment, of blood pressure responses to epinephrine, nicotine, carotid occlusion, and vagal excitation were recorded by a manometer inserted in the femoral artery. In rabbits the gastrocnemius contractions, elicited by sciatic nerve stimulation, were recorded before and after treatment.[3]

From this general screening many compounds belonging to the thymol derivatives were shown to exert a spasmolytic action against nicotine on the isolated jejunum, a hypotensive effect and an inhibiting activity on the nictitating membrane. None of these compounds antagonized *in vivo* the epinephrine action: its contracting effect on the nictitating membrane was unchanged and its blood pressure effect was enhanced. The blood pressure responses to nicotine, carotid occlusion, and vagal stimulation were reduced considerably. Among guaiacol derivatives the only active substances were **15** and **17**. The results for the most interesting compounds are reported in Table II.

TABLE II[a]

	Nict membrane response to stim[b]					
	Preganglionic		Postganglionic		Arterial	Relaxing effect on isolated gut ED_{50}
No.	During treatment	After treatment	During treatment	After treatment	blood pressure fall[c]	against nicotine, μg/ml[d]
4	++	−	++	−	+++	1.1
10	++	+	−	+	+++	1.5
22	+	+	+++	+	+++	4.5
24	++	++	++	++	++	2.5
36	++	+	++	+	+++	2.8

[a] Mean results of two to three experiments. [b] +, ++, +++ = relaxation, respectively, of 10–20, 30–50, and 100% of initial values; mean duration 20 min. − = no relaxation. Stimulation trains of maximal electrical impulses (10–12 cps, 3-msec duration for 2 min, at intervals of 3 min). [c] +, ++, +++ = decrease of blood pressure up to 30, 60, and >60% of initial values, respectively; mean duration ~30 min. Observations on normal or atropinized cats and dogs. [d] Concentration reducing by 50% the maximum nicotine contraction of guinea pig isolated gut.

For these compounds a further series of experiments was performed with the aim of testing their effect (a) on the blood pressure of hypertensive rats;[4,5] (b) on the blood pressure of pentobarbitalized dogs, in which the systemic arterial blood pressure was recorded together with that of a local and peripheral circulatory district (posterior leg);[6] (c) on isolated rabbit hearts (Langendorff) injected with 10–1000 μg or perfused by Krebs solution to which 1–500 μg/ml of the tested substances were added; (d) on the isolated vessels of rabbit ear[7] (**4** and **22** only).

The results of this group of experiments were the following. (a) All compounds of Table II, though hypotensive on the normal anesthetized animals, were unable to decrease the blood pressure of hypertensive rats. (b) Doses equal to one-tenth of the minimal amount acting intravenously as systemic hypotensives gave, when intraarterially injected,[6] substantial vasodilation lasting about 15 min in the vascular bed of the posterior leg, without affecting the systemic pressure; the local and general hypotensive effect were not abolished in previously atropinized animals. (c) In the isolated hearts the beats were unchanged in frequency and amplitude in the entire range of employed doses. (d) Both a vasodilating and an adrenolytic effect on the vessels of the isolated rabbit ear were obtained with **4** and **22** in a range of from 10 to 500 μg/ml of perfusing fluid.

On the basis of these results the mechanism of the observed hypotension does not appear as a simple one. The hypotensive effect can be related partially to a moderate ganglionic blocking activity, as shown by the decrease of the contractions of the preganglionically stimulated nictitating membrane. This mechanism, in spite of the fact that the contractions of the second postganglionically stimulated membrane were equally diminished, is supported by those modifications of the blood pressure response to several agents (increase for epinephrine; decrease or suppression for nicotine, carotid reflex, and vagal stimulation) and by the inhibiting effect on the nicotine contractions of the isolated gut, which are peculiar to all ganglionic blocking agents.

A point of attack at the effector level must also be postulated: the contractions of the nictitating membrane were decreased when postganglionically stimulated; the smooth muscles of blood vessels relaxed appreciably, as shown by the localized vasodilation in a vascular district of a living animal not accompanied by a systemic hypotension; and finally, for two compounds (**4** and **22**), a vasodilating effect was seen on the vessels of the isolated rabbit ear.

A component of the hypotensive effect due to an impairment of the cardiac function is to be excluded, on the basis of the blood pressure and the isolated rabbit heart records. In these the heart beats were unchanged in frequency and amplitude even at concentrations of the drugs in the perfusion fluid well above those acting intravenously as hypotensive in the living animals (rabbits, cats, dogs). In the whole animal an adrenolytic as well as a pure vagal effect must also be excluded, since the epinephrine responses were not abolished or diminished after treatment and the activity of the tested substances was not suppressed after atropinization.

The hypotension and the local vasodilation as well as the decrease in the contractile response of the nictitating

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membrane elicited by postganglionic stimulation seem then to be due not only to a moderate inhibition at the level of the ganglionic synapses but also to a relaxing effect directly exerted by the injected substances on the smooth muscular fibers of the vascular walls and nictitating membrane.

Substituted Heteroaromatic Anthranilic Acids with Antiinflammatory Activity

E. FALCH, J. WEIS, AND T. NATVIG

Research Division, Pharmacia AS, Copenhagen-Vanløse, Denmark

Received November 30, 1967

The pronounced pharmacological activities of N-(2,3-dimethylphenyl)anthranilic acid (mefenamic acid)[1] and of other substituted anthranilic acids[2,3] have stimulated other workers to prepare and evaluate numerous analogs of this class of compounds for their analgetic and antiinflammatory properties. Sutton and Birnie[4] described the synthesis of 1-carboxy-8-trifluoromethylphenothiazine, an active tricyclic sulfur analog of N-(3-trifluoromethylphenyl)anthranilic acid (flufenamic acid). Recently, series of anilinopyridinecarboxylic acids[5] and 4-anilinopyrimidine-5-carboxylic acids[6] have been prepared, and significant antiinflammatory activity has been established for several members of each group of compounds.

These results suggest that the anthranilyl ring of mefenamic acid may be subjected to considerable manipulation without seriously affecting the activity. We wish to report the synthesis and pharmacology of novel anthranilic acids containing heteroaromatic N-substituents. Only a few compounds of this type have previously been reported in the literature.[7]

Chemistry.—The majority of the compounds listed in Tables I–III were prepared by the reaction of appropriately substituted chloro heterocycles with anthranilic acid in hydrochloric acid in a manner similar to that employed by Banks[8] for the preparation of N-(substituted pyrimidinyl)anilines. Alternatively, substituted methylthio heterocycles were treated with anthranilic acid in alkaline solution (**18**, Table II, and **23**, Table III).

N-[5-(4-Carboxy-2,6-dihydroxypyrimidinyl)]anthranilic acid (**20**) was prepared under Ullmann conditions from *o*-bromobenzoic acid and 5-amino-4-carboxy-2,6-dihydroxypyrimidine.

When 2-chloro-4-diethylamino-6-methylpyrimidine and anthranilic acid were heated in dilute HCl, the dihydrochloride of the anilinium salt of **19** was isolated in 45% yield. Under the influence of the 4-diethylamino substituent of the pyrimidine ring, extensive decarboxylation of the anthranilic acid had occurred. This effect was not observed during the preparation of the related *s*-triazine derivatives under the same conditions.

With few exceptions the intermediate chloro- and methylthio heterocycles are known compounds. The preparation of the new compounds of this type by established procedures is described in the Experimental Section. A convenient and efficient method for the synthesis of 5-chloropyrimidines was developed. 5-Chloro-2,6-dimethyl-4-hydroxypyrimidine had previously been prepared by the condensation of acetamidine and ethyl α-chloroacetoacetate.[9] We obtained the compound in high yield by direct chlorination of 2,6-dimethyl-4-hydroxypyrimidine with aqueous sodium hypochlorite. 5-Chloro-4,6-dimethyl-2-hydroxypyrimidine was prepared by the same method, which appears generally applicable to hydroxypyrimidines.

Biological Activity.—The compounds were screened for their antiinflammatory and analgetic activity by the following procedures: yeast edema test in mice,[10] kaolin edema test in rats,[11] and by a modified mouse-writhing test.[12]

Groups of ten white male NMRI mice were dosed orally 30 min prior to the injection of 0.02 ml of a 2% suspension of bakers yeast into the plantar surface of the left hind paw of each animal. After 3 hr the mean per cent weight increase of the inflamed paws was compared with that obtained in the control group, and the results are expressed as per cent inhibition.

The kaolin edema was induced by injection of 0.1 ml of a 10% suspension of kaolin into the left hind paw of groups of ten male Wistar rats immediately after the oral administration of the test compound. The degree of swelling was measured volumetrically 5 hr later and the results are expressed as per cent inhibition of swelling compared to the control group. A fixed oral dose of 300 mg/kg was employed in these two procedures.

In the writhing test for analgesia groups of five male NMRI mice were injected intraperitoneally with 0.2 ml of a 0.75% aqueous acetic acid solution and the writhings for the whole group were counted during the following 20 min. A fixed oral dose of 100 mg/kg of the test compound was given 1 hr prior to the experiment and the reduction in writhings was recorded as per cent inhibition compared with the control group. If the fixed dose resulted in more than 50% inhibition, lower doses were tested, and ED_{50} values were calculated.

In the yeast edema procedure the compounds **3**, **4**, **6**, **7**, **15**, **21**, and **22** showed activities ranging from 12–36% inhibition. Similar effects could be obtained with 50–200 mg/kg of acetylsalicylic acid, while a dose of 20 mg/kg of mefenamic acid resulted in 25% inhibition. The compounds **3**, **5**, **14**, **15**, and **24** caused inhibition of the kaolin edema ranging from 23–57% corresponding to the results obtained with 10–50 mg/kg of phenylbutazone or 25–100 mg/kg of mefenamic acid. In the

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