Skeletal Muscle Stimulants. Substituted Benzoic Acids

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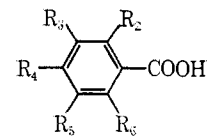
A number of hydrocarbon- and halogen-substituted benzoic acids have been found to produce myotonic symptoms in animals similar to those produced by some veratrum alkaloids. 3-Chloro-2,5,6-trimethylbenzoic acid (**23**) was the most active. Structure–activity relationships pertaining to the myotonic effects are discussed.

As a result of biological screening, a number of substituted benzoic acids have been observed to produce a peculiar syndrome in animals which manifests itself in spasms of the extremities, usually triggered by exertion. The overt symptom is not unlike the clinical symptoms in myotonia congenita (Thomsen's disease). Studies on curarized animals with direct stimulation demonstrated a prolongation of muscle contraction accompanied by repetitive EMG afterdischarge. This effect on the skeletal muscle is similar to that reported for some veratrum alkaloids, phenanthrene-9-carboxylic acid, 2,4-dichlorophenoxyacetic acid, etc.,[1] but none is as potent or selective for skeletal muscle as our more active compounds. A description of the screening test and detailed biology of our most active compound, 3-chloro-2,5,6-trimethylbenzoic acid (**23**), is reported elsewhere.[2]

A material synthesized by Newman, *et al.*,[3] and reported by them to be 2-chloro-3,5,6-trimethylbenzoic acid (**24**) was very active in our test and much of our early work was done on this material. It was subsequently found, by nmr spectroscopy and gas–liquid partition chromatography (glpc) to contain up to 45% of the isomeric acid **23**. Repeated attempts to separate these compounds by crystallization, sublimation, and column chromatography failed, but they can be separated by glpc, preferably as the methyl esters. A more practical method for the separation of substantial quantities of **23** and **24** was devised as shown in Scheme I.

Newman's proof of structure[3] was conclusive for the reported structure **24**, but since the yields of his derivatives were not quantitative, the presence of the other isomer could not be detected. The structure of this isomer (**23**) was established as follows (Scheme I). Nmr measurements on the mixture showed only two peaks in the aromatic region at about δ 7.18 and 7.27. If either component had contained a hydrogen *ortho* to the carboxyl there would have been a peak further downfield.[4] Hydrogenolysis of the mixture selectively removed the chlorine from the *m*-chloro acid (**23**). That the chlorine was removed from **23** rather than from **24** was shown by the isolation of 2,3,6-trimethylbenzoic acid (**17**) whose melting point agreed with the literature and whose nmr showed two adjacent hydrogens.[4]

TABLE I: PHARMACOLOGY



No.	Ring substitution	Lethality[a] (LD_{50})	Myotonic act.[b] (ED_{50})
1	2-CH_3	422	>100
2	3-CH_3	562	>100
3	2,3-$(CH_3)_2$	1000	142
4	2,4-$(CH_3)_2$	>1000	>200
5	2,5-$(CH_3)_2$	>1000	57
6	2,6-$(CH_3)_2$	178	72
7	3,4-$(CH_3)_2$	316	>200
8	3,5-$(CH_3)_2$	750	>100
9	3,5-$(CF_3)_2$	100	>100
10	2-CH_3, 3-Cl	>1000	178
11	2-CH_3, 6-Cl	422	80
12	2,5-Cl_2	237	100
13	2,6-Cl_2	316	112
14	3,5-Cl_2	237	178
15	2,5-Br_2	178	112
16	3,5-Br_2	562	>200
17	2,3,6-$(CH_3)_3$[c]	>1000	21.9
18	2,4,5-$(CH_3)_3$	316	100
19	2,4,6-$(CH_3)_3$	562	>100
20	2,3,6-Cl_3[d]	178	89
21	2,3,5-I_3	562	>50
22	2,3,5,6-$(CH_3)_4$	750	6.3
23	3-Cl, 2,5,6-$(CH_3)_3$[e]	562	4.2
24	2-Cl, 3,5,6-$(CH_3)_3$[e]	750	7.2
25	3,5-[CH$(CH_3)_2$]$_2$, 2,6-$(CH_3)_2$[c]	422	>100
26	2,3,4,5-Cl_4	562	>100
27	2,3,5,6-Cl_4	237	25
28	2,3,4,5,6-$(CH_3)_5$[c]	>200	20
29	2,3,4,5,6-Cl_5	178	32
30	2,3,4,5,6-F_5	178	100
31	Phenanthrene-9-carboxylic acid	>1000	60.3
32	Anthracene-9-carboxylic acid	750	8.0
33	1,2,3,4,5,6,7,8-Octahydroanthracene-9-carboxylic acid	...	46.7

[a] Compounds were administered to mice intraperitoneally. The values (mg/kg) are approximations with an accuracy of about +100% to −50%. [b] Compounds were suspended in 0.25% methylcellulose solution and injected intraperitoneally into mice. Doses were advanced in 0.5-log intervals with four animals at each dose. Median doses were calculated by the method of Spearman and Karber (D. J. Finney, "Statistical Method in Biological Assay," Hafner Publishing Co., New York, N. Y., 1952, p 524). [c] See Experimental Section for method of preparation. [d] Sixty per cent material from Heyden Newport Chemical Corp. was purified by the method of J. Muir, British Patent 909,216 (1962); *Chem. Abstr.*, **58**, 4476e (1963). [e] The authors are indebted to Dr. Gerald Bakker, Earlham College, for a sample of this acid.

The isomer resistant to hydrogenolysis was easily separated and nmr showed it to be the component having a single peak at δ 7.18 (no H *ortho* to the COOH)

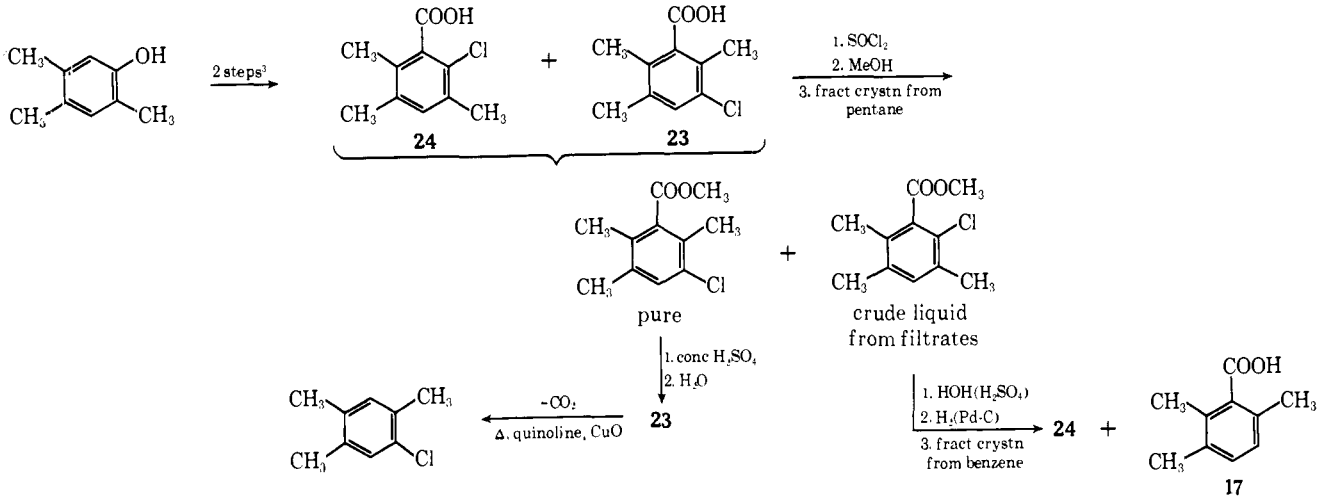
(1) R. G. Smith, *J. Pharmacol. Exp. Ther.*, **54**, 87 (1935); N. L. R. Bucher, *Proc. Soc. Exp. Biol. Med.*, **63**, 204 (1946); O. Krayer and G. H. Acheson, *Physiol. Rev.*, **26**, 383 (1946).

(2) A. H. Tang, L. A. Schroeder, and H. H. Keasling, to be published.

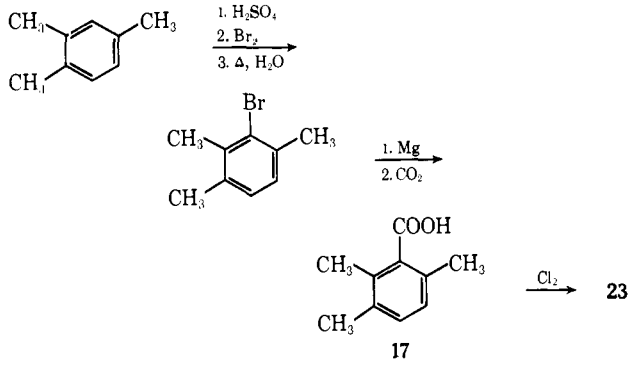
(3) M. S. Newman, D. Pawellek, and S. Ramachandran, *J. Amer. Chem. Soc.*, **84**, 995 (1962).

(4) For example, 2,3,6-trimethylbenzoic acid (**17**), in the aromatic region, shows an AB pattern at about δ 7.0 and 7.1 (J_{AB} = 8 cps) but 2,4,5-trimethylbenzoic acid (**18**) shows two singlets at δ 7.09 and 7.71.

SCHEME I



SCHEME II



and must therefore be **24**, the structure proved by Newman.[3] That the chlorine in **23** was in the 3 rather than the 4 position was shown by decarboxylation of **23** to give 1-chloro-2,4,5-trimethylbenzene (melting point agrees with the literature and nmr shows *p*-hydrogens, singlets at δ 6.90 and 7.04). The structure of **23** was confirmed through synthesis (Scheme II). 2,3,6-Trimethylbenzoic acid, prepared by carbonation of the Grignard reagent[5] from 2-bromo-1,3,4-trimethylbenzene,[6] was chlorinated. As expected the chlorine entered predominantly *meta* to the carboxyl group.

Pharmacology.—The typical myotonic symptoms produced by these benzoic acids in mice consist of a temporary rigid extension of the hind legs when the animal is disturbed. At higher doses, these extensor spasms may occur continuously. The overt appearance is readily distinguishable from convulsion caused by central stimulation. The LD_{50}'s and ED_{50}'s for myotonic symptoms are listed in Table I. Except as noted, these compounds were obtained from commercial sources or prepared by published procedures. A large number of other close analogs of the active compounds were also tested but were found inactive under the conditions of the test. These include esters and amides of active acids, analogous sulfonic acids, and compounds containing an acetyl in place of the carboxyl group. Other groups such as OH, OR, NH_2, $CONH_2$, or additional COOH on the benzene ring invariably proved detrimental to the myotonic activity in mice.

(5) H. A. Smith and J. A. Stanfield, *J. Amer. Chem. Soc.*, **71**, 81 (1949).

(6) G. Lowe, F. G. Torto, and B. C. L. Weedon, *J. Chem. Soc.*, 1855 (1958).

A study of Table I shows that most of the potent compounds for myotonic activity have Cl or CH_3 substitutions at 2,3,5,6 positions (**22**, **23**, **24**, **27**). Larger alkyl groups (**25**, **33**) or substituents at the 4 position (**18** and **19** *vs.* **17**; **26** *vs.* **27**; **4** and **7** *vs.* **3**, **5**, and **6**; **28** *vs.* **22**; **31** *vs.* **32**) yield compounds with weaker activity. Comparison of **22** *vs.* **25** and **32** *vs.* **33** suggests that a relatively flat molecule is more favorable for myotonic activity. The weaker activity of 1,2,3,4,5,6,7,8-octahydrophenanthrene-9-carboxylic acid compared to its fully aromatic parent compound (**31**) has been previously observed.[1] Finally, myotonic activity does not seem to bear any relationship to the potency for lethality.

Experimental Section[7]

Mixture of 2-chloro-3,5,6-trimethylbenzoic acid (24) and 3-chloro-2,5,6-trimethylbenzoic acid (23) was prepared in two steps from 2,4,5-trimethylphenol as described by Newman, *et al.*[3] In the work-up the 4-trichloromethyl-2,4,5-trimethyl-2,5-cyclohexadienone was chromatogaphed instead of distilled using silica gel and eluting with CH_2Cl_2. Some lots of the final mixed acids were sublimed at 110–120° (0.2 mm). These procedures removed tars and other impurities but effected little if any separation of the isomeric acids. The product was finally crystallized from benzene giving white crystalline material, mp ~154–160°. By nmr and glpc (as the Me ester prepared with CH_2N_2) various lots were found to consist of 55–70% of **24** and 30–45% of **23**. A sample kindly supplied by Dr. Newman[3] was found by nmr also to be a similar mixture of these two acids.

Methyl 2-Chloro-3,5,6-trimethylbenzoate and Methyl 3-Chloro-2,5,6-trimethylbenzoate.—A solution of 59.4 g (0.3 mole) of the above mixture (55% **24** and 45% **23**) in 200 ml of C_6H_6 and 150 ml of $SOCl_2$ was refluxed for 4 hr. The solvent was removed under vacuum, and C_6H_6 was added and removed giving the acid chloride as a pale yellow oil. This was dissolved in C_6H_6 (200 ml) and slowly added, with stirring, to 600 ml of MeOH and 18 ml (0.33 mole) of dry pyridine. After refluxing for several hours and removing the solvent, the residue was dissolved in ether, washed (H_2O, dilute HCl, dilute NaOH, H_2O), and dried (Na_2SO_4). After filtration and removal of the ether the product was distilled giving 61.3 g (96%) of colorless liquid, bp 80.5° (0.005 mm), which soon partly solidified, fp ~25–39°. *Anal.* ($C_{11}H_{13}ClO_2$) C, H, Cl.

Small samples of this mixture in $CHCl_3$ were repeatedly injected into a 2-m glpc column packed with 10% Carbowax 20M

(7) Melting points were taken in capillary tubes with a partial immersion thermometer. Calibration of the apparatus against standard compounds showed no need for correction. Ir spectra on all new compounds were in accordance with the proposed structures. Nmr spectra were taken on a Varian A-60 instrument. Where analyses are indicated only by symbols of the elements or functions, analytical results obtained for these elements or functions were within ±0.4% of the theoretical values.

on Diatoport-S 80–100 mesh at 125°. The fractions were collected in Dry Ice cooled traps yielding 77 mg of a liquid and 60 mg of a crystalline solid, mp 67.5–68.5°.

A 48-g sample of the partly solid mixture of Me esters was filtered at room temperature. The crystals were washed with cold pentane and recrystallized three times using about 50 ml of pentane for each crystallization and cooling to −2°. This gave 3 g of white crystals, mp 67–68°. A mixture melting point with the crystalline sample separated by glpc above gave no depression (mmp 67.5–68.5°). By reworking the filtrates a total of 11.4 g (53% of the methyl 3-chloro-2,5,6-trimethylbenzoate known to be present) was obtained having mp 65–67° or higher. Nmr in $CDCl_3$ indicated only one isomer, showing five singlets at δ 7.20, 3.90, 2.25, and 2.15. *Anal.* ($C_{11}H_{13}ClO_2$) C, H, Cl.

3-Chloro-2,5,6-trimethylbenzoic Acid (23).—A mixture of 11.1 g (0.0522 mole) of methyl 3-chloro-2,5,6-trimethylbenzoate and 50 ml of concentrated H_2SO_4 was well shaken and the resulting nearly colorless solution was allowed to stand for 1 hr. It was then poured into 400 ml of ice water and after standing for 1 hr the white solid was collected, well washed (H_2O), dried, and recrystallized from C_6H_6 giving 10.6 g (100%) of white crystals, mp 162–164°. Nmr in $CDCl_3N(CD_3)_2$ indicated only one isomer showing four singlets at δ 7.28, 2.30, 2.25, and 2.20. *Anal.* ($C_{10}H_{11}ClO_2$) C, H, Cl.

2-Chloro-3,5,6-trimethylbenzoic Acid (24).—The liquid ester from the glpc separation above was mixed with 1 ml of concentrated H_2SO_4, shaken for 15 min, and diluted with H_2O. The acid was extracted with ether, washed (H_2O), and dried (Na_2SO_4). Filtration, concentration, and crystallization from C_6H_6 gave 35 mg of white crystals, mp 170.5–171.5°.

Crude filtrates from the above crystallizations of the methyl esters from which no more pure ester could be easily crystallized were evaporated to dryness and mixed with 150 ml of concentrated H_2SO_4. After standing for 4 hr the solution was poured into ice water giving 26.7 g of white solid. This was dissolved in 230 ml of EtOH and hydrogenated for 7 hr with 4 g of 30% Pd–C at 3.5 kg/cm² and 80–90°. After filtration and removal of the solvent the residue was dissolved in dilute NH_4OH, washed with ether, and acidified. The crude acid was extracted with ether, washed (H_2O), and dried (Na_2SO_4). Filtration and evaporation gave 20.6 g of white solid which was recrystallized twice from C_6H_6 yielding 12.8 g (53% of the 2-chloro-3,5,6-trimethylbenzoic acid known to be in the original mixture) of white crystals, mp 171–172.5°. A mixture melting point with the acid obtained from the liquid ester separated by glpc above gave no depression (mmp 171–172°). *Anal.* ($C_{10}H_{11}ClO_2$) C, H, Cl.

2,3,6-Trimethylbenzoic Acid (17).—A hydrogenolysis similar to the above was carried out on 19.6 g of a mixture of acids prepared by the Newman procedure[3] and found by nmr to be a mixture of 45% **23** and 55% **24**. After crystallizing out the acid **24**, the benzene filtrates were fractionally crystallized from C_6H_6 and hexane to isolate the more soluble component. A small sample was thus obtained of pure 2,3,6-trimethylbenzoic acid, mp 109–111° (lit.[8] mp 110.5–112°). This was found to be pure by glpc, ir, and nmr. *Anal.* ($C_{10}H_{12}O_2$) C, H.

Decarboxylation of 3-Chloro-2,5,6-trimethylbenzoic Acid.—A mixture of 1.0 g (0.005 mole) of 3-chloro-2,5,6-trimethylbenzoic acid (**23**), 0.2 g of CuO, and 3.4 ml of quinoline was heated and slowly distilled from a bath at 235–255° during 6.5 hr. The distillate was dissolved in pentane, and washed (dilute HCl, H_2O, dilute NaOH, H_2O). The pentane solution was dried ($CaCl_2$ and Drierite), filtered, and distilled. After removing the pentane the residue was distilled at a bath temperature of 100–130° (12 mm) giving 0.66 g (85%) of white solid, mp 59–66°. This was sublimed at 50–65° (12 mm) giving 0.55 g of crystals, mp 69–71° (lit.[3] for 1-chloro-2,4,5-trimethylbenzene, mp 72.5–73.5°). Nmr and ir are in accordance with the proposed structure. *Anal.* ($C_9H_{11}Cl$) C, H, Cl.

2,3,6-Trimethylbenzoic Acid[9] (17) from Pseudocumene.—2-Bromo-1,3,4-trimethylbenzene was prepared by the method of Lowe.[6,9] Grignard reagent was prepared from 199.1 g (1.0 mole) of this material, 36.5 g (1.5 g-atoms) of Mg, and 750 ml of THF. The reaction was started with 2.5 ml of 1,2-dibromoethane and a small crystal of I_2. The solution was slowly decanted with vigorous stirring into 2 kg of crushed Dry Ice and 1.5 l. of absolute ether. After standing overnight the mixture was decomposed with ice and HCl and the product was extracted with ether. The acid was extracted into aqueous NaOH which was washed (ether) and acidified with HCl. The oily acid was extracted with ether, washed (H_2O), and dried (Na_2SO_4). Filtration and removal of the solvent gave 120 g of white solid which was recrystallized from hexane yielding 68 g (44% of white crystals, mp 110–111.5°. More could be obtained from the filtrates. A mixture melting point with the acid obtained by hydrogenolysis of the chloro acids above showed no depression (mmp 109.5–112°).

3-Chloro-2,5,6-trimethylbenzoic Acid (23) by Chlorination.—To a solution of 1.64 g (0.01 mole) of 2,3,6-trimethylbenzoic acid (**17**) in 25 ml of AcOH was added 7.2 ml (0.011 mole) of a 1.53 *M* Cl_2 solution in CCl_4. The yellow solution was sealed in a heavy glass pressure tube and heated for 16.5 hr in a steam bath. After cooling, the nearly colorless solution was evaporated *in vacuo* and water was added. The crude product was extracted with ether, washed (H_2O), and evaporated. C_6H_6 was added and evaporated under vacuum giving 2.1 g of white solid, mp 118–145°. The ir spectrum was practically identical with that of **23**. Recrystallization from 15 ml of benzene gave 0.53 g (27% of white crystals, mp 152–159°. Two more crystallizations from benzene gave 0.21 g of white crystals, mp 159–162°. A mixture melting point with **23**, above, showed no depression.

1-Bromo-3,5-diisopropyl-2,6-dimethylbenzene.—A solution of 190.3 g (1 mole) of 4,6-diisopropyl-1,3-dimethylbenzene[10] in 200 ml of CCl_4 containing a few crystals of $FeBr_3$ and of I_2 was brominated at room temperature during 7 hr with 168 g (1.05 moles) of Br_2 in 100 ml of CCl_4. After standing overnight the solution was washed (H_2O, dilute NaOH, H_2O) and dried ($CaCl_2$). After removing the solvent the oil was mixed with a solution of 10 g of Na in 200 ml of EtOH, refluxed for 1 hr, and allowed to stand overnight. Water was added, and the product was extracted (ether) and dried ($CaCl_2$). After filtration and removal of the solvent the product was distilled twice through an 85-cm, helices-packed column yielding 183 g (68%) of colorless liquid which solidified in the receiver, bp 118–120° (2.5 mm), fp 54.5–57°. Glpc on a column packed with Carbowax 20M on Diatoport-S indicated this was 99% pure. A middle fraction pressed between filter papers had mp 59–61°. Nmr in $CDCl_3$ indicated that the bromine had entered, as expected, in less hindered position between the methyl groups: δ 7.12 (s, 1), 3.20 (septet, 2, J = 7 cps, H_2), 2.46 (s, 6), and 1.23 ppm (d, 12, J = 7 cps, H_2). *Anal.* ($C_{14}H_{21}Br$) C, H, Br.

3,5-Diisopropyl-2,6-dimethylbenzoic Acid (25).—Grignard reagent was prepared from 80.7 g (0.3 mole) of 1-bromo-3,5-diisopropyl-2,6-dimethylbenzene, 9.72 g (0.4 g-atom) of Mg, and 175 ml of THF. The reaction was started with 0.5 ml of 1,2-dibromoethane. The solution was poured slowly, with vigorous stirring, into a very large excess of crushed Dry Ice in absolute ether. After standing until the excess Dry Ice evaporated, the mixture was decomposed with dilute HCl and the ether solution was washed (H_2O) and extracted with dilute NaOH. The basic aqueous solution was washed (ether) and acidified with HCl giving 59.3 g of crystalline acid, mp 196–204°. Recrystallization from 600 ml of methylcyclohexane yielded 51 g (72%) of white crystals: mp 206–208°; nmr ($CDCl_3$), δ 11.21 (s, 1), 7.24 (s, 1), 3.17 (septet, 2, J = 7 cps, H_2), 2.36 (s, 6), and a doublet at 2.23 (d, 12, J = 7 cps, H_2). *Anal.* ($C_{15}H_{22}O_2$) C, H.

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(8) L. I. Smith and C. L. Agre, *J. Amer. Chem. Soc.*, **60**, 652 (1938).

(9) This material distilled smoothly at 82° (5 mm) and appeared pure by glpc. However, nmr in $CDCl_3$, in addition to the singlets at δ 6.95, 2.36, and 2.25, showed small peaks at δ 7.25 and 2.08 which may be due to the 6-bromo isomer.

(10) From Aldrich Chemical Co., Milwaukee, Wis.