[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF THE UNIVERSITY OF CALIFORNIA]

Branched-Chain Fatty Acids. XXXVI. Synthesis of Three Methyltetracosanoic Acids[1]

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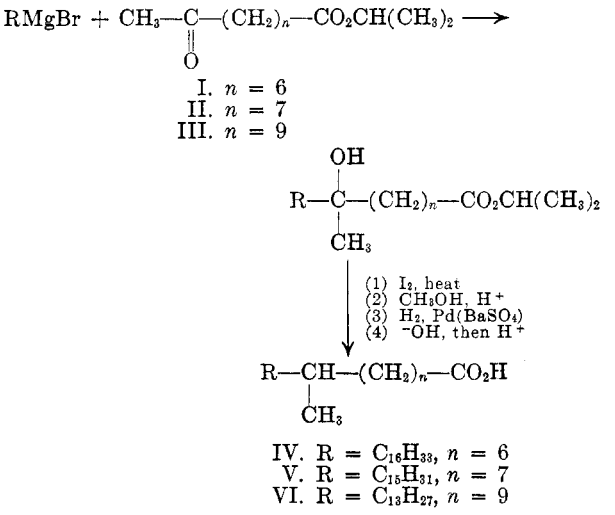
In view of the physiological response to 10-methyltetracosanoic acid, which resembles that of C_{27}-phthienoic acid from tubercle bacillus, there have been synthesized three methyltetracosanoic acids with branches near the 10-position: 8-, 9-, and 11-methyltetracosanoic acids. The syntheses were accomplished *via* the hydroxy ester resulting from reaction of an *n*-alkylmagnesium bromide with an appropriate *sec*-butyl keto ester.

Intraperitoneal injection into rabbits of C_{27}-phthienoic acid, isolated from tubercle bacills,[2] has been found[3] to induce formation of typical tubercles, as had been reported earlier[4] for the mixture of higher molecular weight acids from the tubercle bacillus. Although C_{27}-phthienoic acid is an α-methyl α,β-unsaturated acid[5] with a total of three branches in the chain and twenty-seven carbon atoms, the remotely related 10-methyltetracosanoic acid also has proved[3,6] to cause intraperitoneal lesions rather closely related to tubercles. Numerous other branched higher molecular weight acids, including 2-, 6-, and 18-methyltetracosanoic acids, did not give lesions[3,6] resembling tubercles but gave lesions whose cytological characteristics were similar to typical foreign body reactions. The 14-methyltetracosanoic acid did give lesions[3] exhibiting many of the characteristics of those resulting from the 10-methyl isomer. Interest thus accrues in the cellular reactions of other methyltetracosanoic acids with the substituent near the 10-position, and the present investigation has been concerned with synthesis[7] of the 8-, 9-, and 11-methyl isomers.

The method chosen for synthesis of the methyltetracosanoic acids utilized reaction of a Grignard reagent with a keto ester (I–III) to yield a hydroxy ester. Dehydration of the hydroxy ester, followed by hydrogenation and saponification, yielded the desired acids (IV–VI). As has been previously



reported,[8] preferential reaction of the Grignard reagent with the keto group of a keto ester is improved by use of hindrance in the ester grouping. In the present work, the *sec*-butyl group was used for esterification. The *sec*-butyl ester was transesterified with methanol after dehydration of the hydroxy ester. This lowers the boiling point and improves the heat stability of the ester, and thus facilitates purification by distillation.

Hydrogenation of the unsaturated esters proved unexpectedly troublesome, partly because there appeared to be present catalyst poisons not removed by distillation, and partly because of the insolubility of the high molecular weight esters. Satisfactory hydrogenation was finally secured by first treating the unsaturated esters with Raney nickel, then hydrogenating at 65° in the presence of palladium on barium sulfate, in ether–acetic acid solvent. Ultraviolet absorption below 220 mμ is a very sensitive criterion for completeness of hydrogenation. Whereas a saturated acid, with no branches near the carboxyl,[9] has λ_{max} at about 210 mμ, with ϵ of about 50, an alkene has a rapidly

(1) This investigation was supported in part by a research grant (E-86) from the National Institutes of Health, U. S. Public Health Service.

(2) J. Cason and G. Sumrell, *J. Biol. Chem.*, **192**, 405 (1951).

(3) H. Husseini and S. Elberg, *Am. Rev. Tuberculosis*, **65**, 655 (1952).

(4) F. R. Sabin, *Physiol. Rev.*, **12**, 141 (1932).

(5) J. Cason and C. F. Allen, *J. Biol. Chem.*, **205**, 449 (1953).

(6) B. Gerstl and R. Tennant, *Yale J. Biol. Med.*, **15**, 347 (1943).

(7) 12-Methyltetracosanoic acid has already been prepared by Stina Ställberg-Stenhagen, *Arkiv Kemi, Mineral. Geology*, **A22**, No. 19 (1946). We are indebted to Dr. Ställberg-Stenhagen for sending us a 1.8-g. sample of the immediate precursor of this acid, 11-keto-12-methyltetracosanoic acid, which was reduced in these laboratories by Dr. Max J. Kalm, using the Huang-Minlon procedure. After crystallization from acetone, there was obtained 1.04 g. of 12-methyltetracosanoic acid, m.p. 43.7–45.5°, re-m.p. 51.6–52.5°.

(8) J. Cason and W. L. Stanley, *J. Org. Chem.*, **14**, 137 (1949).

(9) J. Cason and G. Sumrell, *J. Org. Chem.*, **16**, 1177 (1951).

rising absorption below 220 mμ, with ε of several thousand at 200 mμ.

The keto esters required for these syntheses were prepared conveniently by reaction[10] of the methylcadmium reagent with appropriate ester acid chlorides, prepared in turn from half esters. Half esters required for synthesis of isomers I and II were prepared by direct partial esterification of suberic and azelaic acids with *sec*-butyl alcohol. This procedure, in which the half ester is separated by fractional distillation, is not suitable for the preparation of the *sec*-butyl half ester of the higher molecular weight hendecanedioic acid, required for synthesis of III. In this instance, the half methyl ester was prepared by partial saponification of dimethyl hendecanedioate, converted to the ester acid chloride, thence to methyl 11-oxododecanoate. The *sec*-butyl keto ester was secured by transesterification of the methyl ester with *sec*-butyl alcohol.

In order to add to data available on methyltetracosanoic acids, each of the acids was carefully purified and also converted to its amide and *p*-bromoanilide.

EXPERIMENTAL[11]

sec-Butyl hydrogen suberate. A mixture of 100 g. (0.575 mole) of suberic acid, 106 g. (1.43 moles) of *sec*-butyl alcohol, 30 ml. of di-*n*-butyl ether and 15 ml. of concentrated hydrochloric acid was heated under reflux for 2 hr., then subjected to fractional distillation. After removal of the more volatile components, there were collected at 7 mm. pressure the following fractions:

(1) Di-*sec*-butyl suberate, b.p. 170–173°, n_D^{25} 1.4328, 28.14 g.

(2) Intermediate, b.p. 173–184°, n_D^{25} 1.4342, 8.54 g.

(3) *sec*-Butyl hydrogen suberate, b.p. 184–186°, n_D^{25} 1.4392, 44.02 g. (34%).

As a test of purity of the half ester, the entirety of Fraction 3 was subjected to titration.

Anal. Calcd. for $C_{12}H_{22}O_4$: equiv. wt., 230. Found: equiv. wt., 236.

sec-Butyl hydrogen azelate.[12] (*a*) Preparation according to the procedure used for the suberate gave 29.5% yield of half ester of b.p. 175–177°/4 mm., n_D^{25} 1.4431.

Anal. Calcd. for $C_{13}H_{24}O_4$: equiv. wt., 244. Found: equiv. wt., 243.5.

(*b*) After di-ester had been accumulated, the procedure was modified by heating under reflux for 2 hr. a mixture of 54.7 g. (0.29 mole) of azelaic acid, 35.7 g. (0.12 mole) of di-*sec*-butyl azelate, 25.9 g. (0.35 mole) of *sec*-butyl alcohol, 15 ml. of di-*n*-butyl ether, and 7.5 ml. of concentrated hydrochloric acid. The yield of half ester was 43.7 g. (61.5%), b.p. 175–178°/4 mm., n_D^{25} 1.4425; and there was recovered 34.2 g. of di-ester, b.p. 159–162°/4 mm., n_D^{25} 1.4352.

6-Oxohendecanedioic acid. The procedure used is a simplification of that originally reported by Sauer[13] and later employed[14] in these laboratories. To a solution of 89.3 g. (0.5 mole) of δ-carbomethoxyvaleryl chloride (prepared from the commercially available half ester with thionyl chloride, b.p. 155.5–156°/7 mm.) in 500 ml. of dry thiophene-free benzene, cooled to 3–5°, there was added with stirring 50.6 g. (0.5 mole) of purified triethylamine (b.p. 89.6–90°) as rapidly as consistent with keeping the reaction temperature below 25°. About 5 min. is required for the addition. When the mildly exothermic reaction had subsided, the temperature of the reaction mixture was raised to 33–35° during about 15 min. After stirring had been continued without further heating for an additional 0.5 hr., the precipitated triethylamine hydrochloride was removed by suction filtration and washed with about 200 ml. of benzene. Solvent was distilled from the combined filtrate at reduced pressure, and the residue was heated under reflux with about 500 ml. of 2*N* aqueous potassium hydroxide until the mixture became homogeneous (about 4 hr.). The cooled solution was extracted with three portions of ether, then acidified to Congo Red. The white precipitate was crystallized once from a minimal amount of hot water to yield 36.6 g. (63.5%) of 6-ketohendecanedioic acid, m.p. 108–109°; lit.,[13] m.p. 109°.

Methyl hydrogen hendecanedioate. The keto di-acid was reduced by the Huang-Minlon procedure as previously described,[14] and the hendecanedioic acid was esterified with methanol and sulfuric acid. A benzene solution of the crude di-ester was extracted with sodium carbonate solution and washed with water, then solvent was removed to leave a residue of di-ester which was used directly for formation of half ester. Crude di-ester (25 g., 0.105 mole) was dissolved by swirling in 116 ml. of standardized 1*N* solution (0.058 mole) of anhydrous barium hydroxide in commercial anhydrous methanol. The flask was closed with a soda-lime tube and allowed to stand for 16–18 hr. at room temperature;[15] the barium salt of the half ester began to precipitate immediately after mixing. The precipitated salt was collected, washed with a few ml. of methanol, then shaken (without drying) with a mixture of 100 ml. of 4*N* hydrochloric acid and 100 ml. of ether until the solid had been decomposed. The half ester obtained from the ether layer was rapidly distilled through the column. After essentially no forerun, the half ester was collected at 165.5–167.5°/2 mm., weight 15 g. (65%), m.p. 43–44°; lit.,[14] b.p. 170–174°/2.5 mm., m.p. 44–46°.

The filtrate from the barium salt of the half ester contains 15–25% of the di-ester used as starting material, and the small residue from distillation consists largely of di-acid; so essentially all the starting material may be recovered.

Preparation of keto esters was by essentially the procedure that has been described[10] for preparation of ethyl 10-ketohendecanoate. As usual with the methylcadmium reagent,

(10) J. Cason, *J. Am. Chem. Soc.*, 68, 2078 (1946).

(11) All melting points are corrected and all boiling points are uncorrected. All distillations, unless otherwise specified, were through a 75-cm. column of the simple Podbielniak type which has been described in detail (J. Cason and H. Rapoport, "Laboratory Text in Organic Chemistry," Prentice-Hall, Inc., New York, N. Y., 1950, pp. 237–45). Microanalyses were by Microanalytical Division, Dept. of Chemistry, University of California. Ultraviolet spectra were determined on a Beckman DU ultraviolet spectrophotometer.

(12) In this preparation we were assisted by Dr. Max J. Kalm.

(13) J. C. Sauer, *J. Am. Chem. Soc.*, 69, 2444 (1947).

(14) J. Cason, P. B. Taylor, and D. E. Williams, *J. Org. Chem.*, 10, 1187 (1951).

(15) Room temperature was in the range 20–25°. In runs in which aliquots were titrated at intervals, it was found that about 95% of the barium hydroxide had been consumed in 16 hr. Stirring is undesirable in this reaction since it encourages further reaction of the insoluble barium salt of the half ester. The procedure described is a simplification of that reported for another half ester by R. Signer and P. Sprecher, *Helv. Chim. Acta*, 30, 1001 (1947). Although this method is quite effective for half esters of dibasic acids with ten or more carbons, it is poor for azelaic acid and fails for lower molecular weight acids. For adipic acid, the salt of the half ester is quite soluble while barium adipate is insoluble; so essentially no half ester results from the reaction.

rapid addition of the acid chloride to a boiling reaction mixture is imperative, for the mixture rapidly sets to an unstirrable mass. Ester acid chlorides were prepared from the half esters with thionyl chloride. Phosphorus pentachloride proved unsatisfactory for preparation of the *sec*-butyl ester acid chlorides; the product of the reaction was a polymeric material, possibly polymeric anhydride resulting after cleavage of the secondary ester. Yields and properties for each keto ester are listed below.

sec-Butyl 8-oxononanoate (I) was obtained in 76.5% yield, based on ester acid chloride, b.p. 145–147°/5 mm., n_D^{25} 1.4365.

Anal. Calcd. for $C_{13}H_{24}O_3$: C, 68.37; H, 10.62. Found: C, 68.43; H, 11.26.

The infrared spectrum of this compound showed minor absorption in the hydroxyl region, which may have been due to an impurity of hydroxy ester arising from reaction of the methylcadmium reagent with the keto group.

The *semicarbazone* was prepared in aqueous alcohol and recrystallized from hexane to yield colorless plates, m.p. 89.5–90°.

Anal. Calcd. for $C_{14}H_{27}O_3N_3$: N, 14.72. Found: N, 14.55.

sec-Butyl 9-oxodecanoate (II) was obtained in 53–60% yields, b.p. 148–150°/4.5 mm., n_D^{25} 1.4363.

Anal. Calcd. for $C_{14}H_{26}O_3$: C, 69.38; H, 10.82. Found: C, 68.92; H, 10.96.

Saponification of a 1-g. sample of the ester and crystallization of the acid from acetone yielded *9-oxodecanoic acid*, m.p. 47.3–48.1°; lit.,[16] m.p. 49°.

Anal. Calcd. for $C_{10}H_{18}O_3$: equiv. wt., 186. Found: equiv. wt., 184.

sec-Butyl 11-oxododecanoate (III). By the cadmium reaction *methyl 11-oxododecanoate* was prepared in 78% yield, b.p. 155–159°/4.5 mm., n_D^{25} 1.4430–1.4435. For transesterification, a 10.32-g. sample of the methyl ester was added to 75 ml. of dry (by azeotropic distillation) *sec*-butyl alcohol containing 10% by weight of anhydrous hydrogen chloride. The mixture was heated at 110° under the column for 2 hr., then excess *sec*-butyl alcohol and hydrogen chloride were removed at reduced pressure. The residue was distilled at 4 mm. pressure to yield 1.8 g. of forerun, b.p. 146–164°, mostly methyl ester, and 8.4 g. (72%) of *sec*-butyl ester, b.p. 164–166°.

Anal. Calcd. for $C_{16}H_{30}O_3$: C, 71.05; H, 11.15. Found: C, 71.41; H, 10.82.

11-Oxododecanoic acid was prepared by saponification of the forerun from the *sec*-butyl ester. Crystallization from hexane yielded white crystals, m.p. 61–61.5°.

Anal. Calcd. for $C_{12}H_{22}O_3$: C, 67.24; H, 10.36; equiv. wt., 214. Found: C, 67.44; H, 10.25; equiv. wt., 213.

Methyltetracosanoic acids were prepared by the general procedure which will be described in detail for the 9-methyl isomer.

9-Methyltetracosanoic acid (V). A Grignard reagent was prepared from 15.6 g. (0.054 mole) of pentadecyl bromide[17] (b.p. 159–160°/5 mm.), and 1.3 g. (0.054 mole) of magnesium in 135 ml. of ether. Ether was then distilled from the solution by heating on a steam bath until distillation became slow, then 150 ml. of dry benzene was added and stirred into the mixture. Finally, the solution of Grignard reagent was added dropwise to a stirred and cooled solution of 8 g. (0.03 mole) of *sec*-butyl 9-oxodecanoate in 50 ml. of benzene. Cooling was in an ice-salt bath, and addition was regulated so that the temperature could be maintained in the range 0–5°. After addition had been completed (about 0.5 hr.) the mixture was stirred at the same temperature for an additional 0.5 hr., then decomposed with ice and 6*N* sulfuric acid. The organic layer was washed with water, 5% aqueous solution of sodium carbonate, water, and saturated sodium chloride solution. After solvent had been distilled, last traces at reduced pressure, the residue was heated with a few iodine crystals at 190–200° for 1 hr. Relatively volatile material, including any unreacted keto ester, was removed at reduced pressure, then the residue was transesterified by heating under reflux for 4 hr. with 50 ml. of anhydrous methanol and 5 g. of concentrated sulfuric acid. The reaction mixture was diluted with water and extracted with benzene. The benzene solution was washed as described above, and the methyl ester was distilled in a Claisen flask; b.p. 225–228°/1 mm., yield 10.52 g. (56.5%). In another run, the yield was 59%.

The white, low-melting unsaturated ester from Claisen distillation was heated under reflux with 2 g. of commercial Raney nickel in a solution with 50 ml. of methanol and 50 ml. of benzene. After the Raney nickel had been removed by filtration and the solvent by distillation, the product was distilled through the column to yield 7.2 g., b.p. 228–232°/3 mm. The distillate was immediately[18] hydrogenated in the presence of 4 g. of commercial 5% palladium on barium sulfate catalyst, in 300 ml. of a 1:1 mixture of anhydrous ether and glacial acetic acid (distilled from permanganate). To promote solubility, the hydrogenation was carried out at 65°. Hydrogenation had been completed in 12 hr. After removal of the catalyst by filtration and the solvent by distillation, the saturated ester was saponified by heating under reflux with 4 g. of potassium hydroxide in 100 ml. of 95% ethanol. The acid was crystallized three times from acetone to yield 4.5 g. of product with the constant m.p. of 50.1–51°. After the melt had re-solidified it re-melted at 58.0–58.8°, or in a range between the two melting points; so this isomer appears polymorphic, as has been reported for the 12-methyl isomer.[7]

Anal. Calcd. for $C_{25}H_{50}O_2$: C, 78.55, H, 13.14; equiv. wt., 382.5. Found: C, 78.50; H, 12.93; equiv. wt., 382.0.

The ultraviolet spectrum, taken in spectroscopically pure hexane, showed the absence of unsaturated acid: ϵ_{210} 55, ϵ_{220} 33.

For preparation of the *amide*, the acid chloride was prepared by use of thionyl chloride from 0.5 g. of acid, and was treated with a large excess of cold concentrated aqueous ammonium hydroxide. The precipitated white solid was crystallized three times from acetone, after which the m.p. was constant at 76–76.5°.

Anal. Calcd. for $C_{25}H_{51}ON$: N, 3.67. Found: N, 3.44.

The *p-bromoanilide* was prepared by a method previously described[19] and crystallized three times from acetone, m.p. 86.8–88°.

Anal. Calcd. for $C_{31}H_{53}ONBr$: N, 2.60. Found: N, 2.55.

8-Methyltetracosanoic acid (IV). The synthesis was carried out with the Grignard reagent from 0.08 mole of refractionated commercial cetyl bromide (b.p. 171.5–172°/5 mm.), which was allowed to react with 0.05 mole of *sec*-butyl 8-oxononanoate. The yield of crude unsaturated methyl ester was 9.4 g. (57%), and the yield of purified acid, m.p. 53–54°, was 6 g. Ultraviolet absorption showed ϵ_{210} 49, ϵ_{220} 31.

Anal. Calcd. for $C_{25}H_{50}O_2$: C, 78.55; H, 13.14; equiv. wt., 382.5. Found: C, 79.07; H, 13.33; equiv. wt., 383.0.

The *amide* had m.p. 81.5–82°.

Anal. Calcd. for $C_{25}H_{51}ON$: N, 3.67. Found: N, 3.71.

The *p-bromoanilide* had m.p. 91–92°.

Anal. Calcd. for $C_{31}H_{53}ONBr$: N, 2.60. Found: N, 2.57.

11-Methyltetracosanoic acid (VI). The Grignard reagent was prepared from 0.05 mole of tridecyl bromide[17] (b.p. 142–143.5°/7.5 mm.) and allowed to react with 0.036 mole

(16) P. van Romburgh, *Proc. Koninkl. Nederland. Akad. Wetenschap.*, **20**, 195 (1911).

(17) Tridecyl bromide and pentadecyl bromide were prepared by the silver salt and bromine reaction according to the procedure described on p. 272 in the reference in footnote (11).

(18) Numerous purification procedures, solvents, and catalysts were explored before the conditions described were found satisfactory for this hydrogenation.

(19) J. Cason, *J. Org. Chem.*, **13**, 227 (1948).

of *sec*-butyl 11-oxododecanoate. The yield of unsaturated methyl ester, b.p. 213–218°/1.5 mm., was 8.46 g. (79%). The purified acid was obtained in a yield of 5 g., m.p. 59–59.5°. Absorption in the ultraviolet showed ϵ_{210} 44, ϵ_{220} 24.

Anal. Calcd. for $C_{25}H_{50}O_2$: C, 78.55; H, 13.14; equiv. wt., 382.5. Found: C, 78.67; H, 13.37; equiv. wt., 377.

The *amide* had m.p. 82–82.5°.

Anal. Calcd. for $C_{25}H_{51}ON$: N, 3.67. Found: N, 3.64.

The *p-bromoanilide* had m.p. 72.5–73°.

Anal. Calcd. for $C_{31}H_{53}ONBr$: N, 2.60. Found: N, 2.43

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Methylated 2-Amino-5-chlorobenzoxazoles

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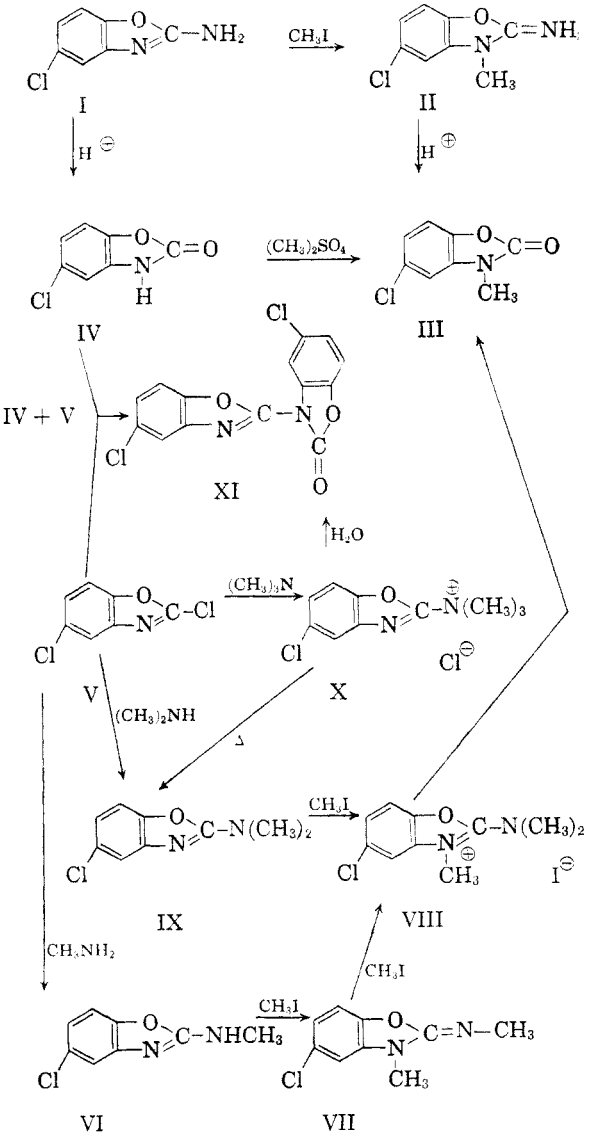
Preparation of all of the mono-, di-, and tri-methylated derivatives of 2-amino-5-chlorobenzoxazole is reported. Structure proofs, interconversions and spectral data of the methylated compounds are described. The two quaternary compounds (VIII and X) proved to be less stable than anticipated. The structures of the products resulting from reactions of the quaternary compounds under mild conditions were proved.

The discovery of the useful muscle-relaxant properties of 2-amino-5-chlorobenzoxazole (I)[1b] stimulated us to investigate compounds of related structure. One series of compounds, the six possible *N*-methylated derivatives of I, forms the subject of this communication.

As with the parent 2-aminobenzoxazole,[2] methylation of I with methyl iodide proceeded to give exclusively the 3-methyl derivative II. Analytical and spectral data were in accord with structure II which was confirmed by acid hydrolysis to 3-methyl-5-chloro-2-benzoxazolinone (III). An authentic sample of the latter compound was prepared for comparison by the dimethyl sulfate methylation of 5-chloro-2-benzoxazolinone (IV).

For the preparation of the other possible monomethyl derivative (VI), 2,5-dichlorobenzoxazole (V)[3] was treated with 25% aqueous methylamine solution.[4] The resultant base VI was isomeric with but different from II and was assigned the structure 2-methylamino-5-chlorobenzoxazole. When monomethyl derivative VI was treated with methyl iodide, there resulted 5-chloro-3-methyl-2-methyliminobenzoxazoline (VII) and further methylation of VII gave the quaternary salt 5-chloro-2-dimethylamino-3-methyl benzoxazolium iodide (VIII). The latter compound upon heating in methanol was transformed readily into 3-methyl-5-



(1) (a) Present address: E. I. du Pont de Nemours and Co., Inc., Camden, S. C.

(1) (b) Flexin® zoxazolamine, McNeil; R. T. Smith, K. M. Kron, W. P. Peak, and I. F. Hermann, *J. Am. Med. Assoc.*, **160**, 745 (1956); E. H. Abrahamsen and H. W. Baird III, *J. Am. Med. Assoc.*, **160**, 749 (1956); W. Amols, *J. Am. Med. Assoc.*, **160**, 742 (1956); M. Rodriguez-Gomez, A. Valdes-Rodriguez, and A. L. Drew, *J. Am. Med. Assoc.*, **160**, 752 (1956).

(2) R. D. Desai, R. F. Hunter, and A. R. K. Khalidi, *J. Chem. Soc.*, 1186 (1934).

(3) Prepared from 2-mercapto-5-chlorobenzoxazole by the method of H. N. McCoy, *Am. Chem. J.*, **21**, 111 (1899); *cf.* L. Katz and M. S. Cohen, *J. Org. Chem.*, **19**, 767 (1954).

(4) This method has been used to prepare substituted 2-aminobenzoxazoles; see, for example, P. Seidel, *J. prakt. Chem.* [2], **42**, 454 (1890).