diarylmethane was recovered by evaporating the hexane extract to constant weight. Identification of these products was made by infrared spectrometic analysis. The results are summarized in Table II

Pyrolysis of Bibenzyls.—(a) Bibenzyl (57.1 g., m.p. 49–50°) was evaporated at 100° and 1 mm. pressure and the vapor stream was pyrolyzed at 940° and 0.04 sec. residence time. The pyrolyzate was condensed at −78° and this was subsequently separated by distillation to afford 1.2 g. (2% yield) of phenyl-*o*-tolylmethane (b.p. 70–75° (0.5 mm.)); 6.3 g. (11% yield) of *trans*-stilbene (b.p. 85–90° (0.5 mm.), m.p. 124°) and 9.1 g. (16% yield) of anthracene (b.p. <90° (0.5 mm.), m.p. 210–212°).

(b) **4,4′-Dimethylbibenzyl (1,2-Di-*p*-tolylethane).**—1,2-Di-*p*-tolylethane (57.8 g., m.p. 79–80°) was vaporized at low pressure and the gas stream was subjected to fast flow pyrolysis. The pyrolyzate was leached with ether. The extract was separated by distillation at atmospheric pressure. The distillate boiling in the range 60–140° was analyzed quantitatively by gas chromatography and the components were identified by mass spectrographic and infrared analysis as benzene, toluene, *p*-xylene, styrene, *p*-ethyltoluene and *p*-methylstyrene. Others[5,19,20] have also reported that these compounds are produced when 1,2-di-*p*-tolylethane and related products are subjected to pyrolysis at low pressure. The ether-insoluble residue was leached with hot toluene and this residue was identified as poly-*p*-xylylene. The toluene extract was evaporated to dryness and admixed with the previous material whose boiling point at atmospheric pressure was greater than 140°. This mixture was separated by distillation under vacuum and subsequent recrystallization as described above. The components were identified by their corresponding m.p. and infrared spectra as unreacted starting material, diarylmethanes, anthracenes and stilbenes. The results are summarized in Table IV.

Pyrolysis of *o*-Xylene.—(a) *o*-Xylene was pyrolyzed at 925°, 0.006 sec. residence time, using the procedure described for *p*-xylene[12] and 1,2-di-*o*-tolylethane (m.p. 50–55°; τ-values 2.99 for phenyl, 7.24 for methylene and 7.77 for methyl) was obtained in 4% yield per pass. The compound was purified further by sublimation and subsequent recrystallization from methyl alcohol to yield white needle-like crystals (m.p. 63–65°).

(b) *o*-Xylene was pyrolyzed at 1000° and 0.023 sec. residence time and anthracene was obtained in 13% yield per pass. The product was purified by sublimation and recrystallization from methanol to afford anthracene in the form of white platelets (m.p. 210–212°).

Acknowledgment.—The authors are indebted to Drs. H. L. Dinsmore and H. F. White, then of the M. W. Kellogg Co., for interpretation of the mass and infrared spectra, and to Drs. F. A. Bovey and G. V. D. Tiers of the Minnesota Mining and Manufacturing Co. for interpretation of the nuclear magnetic resonance spectra.

(19) R. S. Corley, H. C. Haas, M. W. Kane and D. I. Livingston, *J. Polymer Sci.*, **13**, 137 (1954).

(20) S. L. Madorsky and S. Straus, *J. Research Natl. Bur. Stds.*, **55**, 223 (1955), R. P. 2624.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF CALIFORNIA, BERKELEY, CALIFORNIA]

Contribution to the Structure of the Ferrichrome Compounds: Characterization of the Acyl Moieties of the Hydroxamate Functions[1,2]

BY THOMAS EMERY AND J. B. NEILANDS

RECEIVED JANUARY 12, 1960

The acyl moieties of the hydroxamate functions of ferrichrome and ferrichrome A have been characterized as three residues each of acetic and *trans*-β-methylglutaconic acid, respectively.

The isolation and general properties of the ferrichrome compounds have been reviewed elsewhere.[3a] These substances and other possibly related products appear to be widely distributed in microbial species. They are characterized chemically by their ability to form stable coördination compounds with ferric ion; biologically some of them, *e.g.*, ferrichrome itself, are distinguished by their potent growth-promoting activity for a number of unicellular organisms. Their widespread distribution and potent physiological activity appeared to justify structural work on these products in spite of the fact that the pure compounds can only be obtained on a milligram scale. Since all of the compounds which exhibit very intense growth-factor activity either contain iron or are capable of complexing this element,[3] it was concluded that the iron-binding center might also be the seat of biological activity. Consequently, it was decided to concentrate our efforts on this part of the molecule.

Ferrichrome is a neutral substance and ferrichrome A contains three carboxyl groups per mole.[3] When the iron was removed from either of these compounds, electrometric titration of the product revealed the presence of three new ionizable groups with average pK_a' of about 9. Concomitant measurements of light absorption in the ultraviolet (250 mμ) showed this pK to be spectrophotometrically operable. Thus, in alkali the ultraviolet absorption was intensified and shifted to the red in a manner somewhat characteristic of keto–enol tautomerism. It was speculated[3] that enolic groupings might be wholly or partly responsible for binding the iron atom, although the intensity of the ultraviolet absorption in alkali was much less than what would be expected for the usual enol.

The problem remained at this level of development until an unknown spot on a paper chromatogram, prepared from an acid hydrolysate of ferrichrome, was identified as hydroxylamine.[3b] A progress curve for the liberation of hydroxylamine showed that the latter was bound too firmly to be present as an oxime. This experiment served to focus attention on the hydroxamic acid grouping as a possible source of NH_2OH in the ferrichrome compounds. That these natural products are indeed ferric hydroxamates is evident from these facts:

1. Prolonged acid hydrolysis of ferrichrome or ferrichrome A liberates 3 moles of "hydroxylamine."

(1) Abstracted from the doctoral dissertation of Thomas Emery, University of California, Berkeley, 1960.

(2) This research was sustained by a grant from the Office of Naval Research.

(3) (a) J. B. Neilands, *Bact. Rev.*, **21**, 101 (1957). (b) The authors are indebted to Mr. B. Burnham for assistance with; this phase of the investigation.

2. When iron is removed from the ferrichromes, three new equivalents of titratable groups appear with pK_a' of approximately 9. This pK, which is entirely absent in the ferric complexes, corresponds favorably to the literature values for hydroxamic acids.[4]

3. The change in the ultraviolet spectra of the iron-free ferrichromes as a function of pH agrees exactly with that of either free or N-substituted model hydroxamic acids. The synthetic hydroxamic acids used for comparison were acethydroxamic acid and N-methylacethydroxamic acid.

4. The spectra and extinction coefficients for the intact ferrichromes and the synthetic ferric hydroxamates are essentially identical at neutral pH. At low pH there is a significant discrepancy in spectral properties. This difference, however, can be resolved by assuming that the three hydroxamate functions are all attached to the same molecule (see below).

5. The ferrichrome compounds are very stable ferric complexes, but the ferrous ion is bound only weakly or not at all. The iron-free compounds react with cupric ion to give a very insoluble green precipitate. These are the expected properties for metal hydroxamates.

6. The magnetochemical data for the ferrichromes are compatible with the proposed ferric hydroxamate structure. Ehrenberg[5] showed these substances to be spin-five so called "ionic" complexes.

7. Periodate reacts with both the iron-free ferrichromes and the synthetic model hydroxamic acids to split the molecules into the acyl and hydroxylamine moieties. The acyl part is liberated as a carboxylic acid and, in the case of ferrichrome, it is acetic acid. The acyl portion of ferrichrome A, on the other hand, is a dicarboxylic acid (see below).

8. Periodate reacts only slowly with the intact ferric complexes, and the iron atom is separated from the molecule during the course of this oxidation.

Ultracentrifugal data[6] show that the ferrichromes are not composed of three separate similar or dissimilar components. In addition, 6 N hydrochloric acid hydrolysates of ferrichrome have always yielded not more than one mole of ornithine,[3a] and we have been unable to detect inhomogeneity in our preparations of iron-free ferrichromes by paper chromatographic analyses.

The hexadentate structure for the ferrichromes imparts an unusual degree of stability to the ferric complex. This augmented stability of the central metal ion can be demonstrated in at least two ways: (a) In very dilute acid the visible absorption band for the ferric acet- and benzohydroxamates was diminished in intensity and shifted approximately 70 mμ to the red concomitant with the appearance of a bright wine color. The ferrichrome compounds, on the other hand, were observed to retain their yellow color even at very low pH. In dilute mineral acid the yellow color faded to a colorless solution through a transient pale violet intermediate. (b) Sodium ethylenediaminetetraacetate is able to bleach only slowly and incompletely the yellow color of the ferrichromes; ferric triacethydroxamate, on the other hand, is instantly and completely decolorized by similar treatment.

The failure of the ferrichromes to develop the characteristic wine color of simple ferric hydroxamates at low pH was the principal reason for our inability to recognize at once the true nature of the substances. In addition, we were unaware that at neutral pH simple ferric hydroxamates are yellow in color. This color transformation has only rarely been mentioned in the literature[7] and is attributed to the formation of 3:1 complexes in neutral solution and lower complexes at acid pH.[7-9] The latter condition is apparently greatly preferred for analytical work since the red color is more specific and, in addition, the low pH tends to keep any excess iron in solution. Unfortunately, most standard works on coördination chemistry[10,11] do not mention the hydroxamic acids and much remains to be discovered about the ligand properties of these compounds.

The iron-free ferrichromes are instantly oxidized by periodate, and the latter reagent would appear to be generally applicable for the characterization of hydroxamic acids. In the case of ferrichrome, the liberated acyl moiety was extracted into ether and characterized by the usual methods as three equivalents of acetic acid. With ferrichrome A, on the other hand, ether extraction of the periodate-treated and acidified solution yielded a crystalline solid containing both the three original carboxylic residues and the three new acid groups generated by oxidative scission of the hydroxamic functions. Automatic electrometric titration[12] suggested this material to be a dicarboxylic acid with neutral equivalent of 72 and elementary analyses supplied the formula $C_6H_8O_4$. The m. p. of 131–135° did not correspond to that of any common unsaturated dicarboxylic acid. The infrared spectrum also indicated the possible presence of unsaturation and the strong absorption band at 217 mμ, typical of alkyl substituted double bonds in conjugation with a carboxyl,[13] substantiated this conclusion. The compound was found to reduce alkaline permanganate. A small sample was reduced with hydrogen and palladium–charcoal to yield a crystalline solid with m.p. 82–84°. Since the latter corresponded reasonably well with the literature value for β-methylglutaric acid (84–85°[14]) and was a few degrees higher than the stated m.p. of the α-methyl isomer (77–78°[15]) the original compound was suspected to be β-methylglutaconic acid (I).

(4) W. M. Wise and W. W. Brandt, THIS JOURNAL, **77**, 1058 (1955).
(5) A. Ehrenberg, *Nature*, **178**, 379 (1956).
(6) Courtesy of Jean Miller and H. K. Schachman.
(7) S. Fiala and D. Burk, *Arch. Biochem.*, **20**, 172 (1950).
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(13) James Cason, personal communication.
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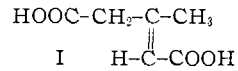


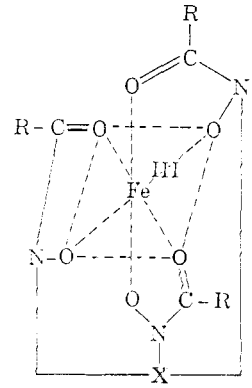
I

Although I apparently has not been previously isolated from natural sources, the fact that Adamson and Greenberg[16] found only the low-melting, mainly *trans* acid to be a precursor of cholesterol would suggest that the natural product should be of similar geometrical configuration. The infrared spectra of the two forms are reported to show only minor variations,[17] and this was confirmed by us through use of synthetic *cis* and *trans* I obtained by opening of ethyl isodehydroacetate.[18] On the other hand, we found that the dimethyl esters of the two forms could be very readily separated by gas chromatography, and by this means we were able to identify the unsaturated dicarboxylic acid obtained from ferrichrome A as pure *trans* I. At this stage there remained only the apparent discrepancy in melting point since the usual literature value given for *trans* I is 115–116°.[18] However, Jackman and Wiley[19] have very recently reported a m.p. of 140° for pure *trans* I. A synthetic specimen obtained through the courtesy of Dr. Wiley was found to melt at 130–135° and showed no depression on admixture with our natural product. Low-melting preparations of I may hence be impure[19] or, alternatively, they may represent a polymorphic crystalline form of pure *trans* I.[18]

Additional support for characterization of the natural compound as a β-methylglutaconic acid was obtained by comparison of the infrared and melting point behavior of its reduction product with that of β-methylglutaric acid. No differences were found.

The content of I in ferrichrome A was determined to be three equivalents per mole through use of properly controlled extinction coefficients of the unsaturated acid at two wave lengths in the deep ultraviolet. In any monohydroxamic derivative of I, the double bond could be either α-β or β-γ with respect to the acyl grouping. In the case of ferrichrome A the argument in favor of the α-β position is: The broad absorption bands of ferrichrome and ferrichrome A with maxima at 425 and 440 mμ and ε of 2895 and 3740, respectively, compare favorably with those for ferric acethydroxamate with a maximum at 425 mμ and ε of 2895 and ferric benzohydroxamate with a maximum at 435 mμ and ε of 4050. These data are for *p*H 7. When ferrichrome A was reduced with hydrogen and palladium–charcoal, the absorption maximum was shifted to 425 mμ and was diminished to the expected extent. The α-β unsaturation would be expected if I were to arise biosynthetically through the carboxylation of β-methyl crotonyl Coenzyme A.[20] The β-dialkyl substitution of the double bond should be acid-weakening,[21] and this in turn should increase the affinity for ferric ion.[8] Garibaldi[22] has shown that the ferrichrome A complex is several orders of magnitude more stable than the corresponding ferrichrome complex.

The hydroxamic acid nature of the ferrichrome compounds has already been mentioned in a preliminary report.[23] From the present discussion it is evident that the iron-binding center of the ferrichromes can be formulated as



Ferrichrome R = $-CH_3$

Ferrichrome A R = $-\underset{\underset{CH_3}{|}}{\overset{\overset{H}{|}}{C}}=C-CH_2-COOH$

The nature of X is under investigation. It cannot be decided, from present knowledge, whether or not the trihydroxamate structure shown is the fundamental requirement for biological activity.

Experimental[24]

Isolation of Ferrichrome and Ferrichrome A.—These compounds were isolated from *Ustilago sphaerogena* fermentations by previously described methods.[3a,25] Ferrichrome was recrystallized once from methanol while ferrichrome A was recrystallized three times from water. Both were dried 12 hours at 100° over P_2O_5, *in vacuo*.

Removal of Iron from the Ferrichromes.—The iron can conveniently be removed from ferrichrome by treatment with strong base. A 10 mg. quantity of ferrichrome was dissolved in 1 ml. of water and 1 ml. of 1 *N* KOH added. The solution was allowed to stand at 0° for 30 minutes and then centrifuged hard. The colorless supernatant was carefully removed from the ferric hydroxide ppt. and immediately adjusted to neutrality with 1 *N* HCl.

The above procedure is ineffective in completely removing the iron from ferrichrome A and hence a different procedure was adopted: 100 mg. of ferrichrome A was dissolved in 5 ml. of water through the addition of 1 *N* KOH to *p*H 7–8. After the addition of 1.25 ml. 1 *N* KCN and 25 mg. of $Na_2S_2O_4$ the stoppered solution was allowed to stand for one hour at room temperature. The now colorless solution was adjusted to *p*H 2 with 6 *N* HCl and ammonium sulfate then added to incipient turbidity. The solution was extracted with benzyl alcohol until a drop of the extract gave no color upon addition of a small crystal of ferric chloride. Care must be taken to avoid carrying over any aqueous phase, the latter containing ferrocyanide, with the organic extracts. To the combined alcohol extracts was added 10 volumes of

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(22) J. A. Garibaldi, Doctoral dissertation, University of California, Berkeley, 1958.

(23) T. Emery and J. B. Neilands, *Nature*, **184**, 1632 (1959).

(24) Melting points uncorrected. Microanalysis by Chemistry Department, University of California, Berkeley. Ultraviolet and infrared spectra were measured with the Process and Instruments and the Baird Associates spectrophotometers, respectively.

(25) J. A. Garibaldi and J. B. Neilands, THIS JOURNAL, **77**, 2429 (1955).

ether and the turbid solution shaken out with water until no more ferric chloride positive material could be extracted. The combined aqueous extracts were adjusted to neutrality with 1 N KOH. Attempts to crystallize the iron-free ferrichromes have not been successful.

The exact ferrichrome content of the solutions was determined through use of the visible absorption spectrum which appeared upon re-addition of iron. A typical assay was as follows: A 0.025 ml. aliquot of the iron-free ferrichrome solution was placed in a 10 ml. volumetric flask containing 5 ml. water. After the addition of 0.01 ml. of a ferric chloride solution containing 5.0 mg./ml. iron the contents of the flask were mixed and made up to the mark with 0.1 M sodium acetate, pH 4.6. A blank was prepared without the ferrichrome solution. The optical density of the solution was read at 425 and 440 mμ for ferrichrome and ferrichrome A, respectively, and the concentration of the complex determined by reference to a standard curve. An additional 0.01 ml. of ferric chloride solution was added routinely to both blank and sample to ensure that iron was in excess. The recovery of ferrichrome compounds by this method was 95–100% of the theoretical quantity.

Hydroxylamine Determination.—A stock solution of crystalline ferrichrome or ferrichrome A was prepared by dissolving a dry, carefully weighed sample in water. The final concentration was approximately 15 to 30 μg./ml. One ml. of the solution was sealed in a Pyrex tube with 1 ml. of 6 N H_2SO_4 and hydrolysed at 106°. Control experiments revealed maximum liberation of NH_2OH after 12 hours. The cooled samples were transferred to graduated tubes and assayed by the method of Csáky.[26] The iodine and arsenite solutions were used at one-half strength in order to avoid precipitation of iodine. Concentrations of hydroxylamine were determined colorimetrically by reference to a standard curve obtained with a freshly prepared solution of reagent grade hydroxylamine hydrochloride. Control experiments were performed using pure hydroxylamine hydrochloride, acethydroxamic acid[4] and benzohydroxamic acid, the latter prepared by the method of Blatt.[27] Recoveries, after 12 hours of hydrolysis, were 82.5, 83.0 and 86.0%, respectively. Ferric iron was found not to interfere. The results are given in Table I.

TABLE I

HYDROXYLAMINE CONTENT OF THE FERRICHROME COMPOUNDS

	Hydroxylamine[a]	
	mμ moles	Moles/mole complex
Ferrichrome	54.2	3.08
17.6 mμ moles	51.7	2.94
Ferrichrome A	53.2	2.87
18.6 mμ moles	54.2	2.92

[a] Values corrected for 84% recovery.

The presence of free hydroxylamine in the hydrolysate was confirmed by paper chromatography. Some difficulty was experienced in chromatographic detection of NH_2OH in the hydrolysates of the intact complexes, probably through interference from iron. The identification was therefore carried out on a solution of the iron-free compound. One-half ml. of solution, containing about 10 mg. iron-free ferrichrome A (for example), was hydrolyzed in 3 N HCl for 12 hr. at 106° in a sealed tube. The excess acid was removed over NaOH and the residue taken up in 0.1 ml. water. Aliquots of 0.01 ml. were chromatographed by the ascending technique on Whatman No. 1 paper in solvent systems containing the following ratios of 95% ethanol and 6 N HCl: (1) 80:20; (2) 60:40; (3) 40:60; (4) 20:80 and (5) with methanol: 6 N HCl, 70:30. The spots were detected by spraying the dried chromatograms with the picryl chloride and ammoniacal acetylmonoxime nickel sprays of Bremner[28] and the tetrazolium spray of Snow.[29] The R_f values are as shown. The tetrazolium and monoxime-nickel sprays yielded red spots while the picryl chloride spray gave orange spots which became yellow-brown upon drying. Better results were obtained when the dried papers were exposed to ammonia vapor before spraying.

Solvent system	NH_2OH	Ferrichrome A hydolysate
1	0.38	0.37
2	.53	.53
3	.69	.67
4	.85	.85
5	.58	.58

pH Dependence of Ferrichrome Spectra.—Aqueous solutions of $2 \times 10^{-4} M$ ferrichrome and ferrichrome A were prepared and the pH adjusted by the addition of 1 N KOH or 1 N HCl. The spectral properties as a function of pH are recorded in Table II.

TABLE II

SPECTRAL PROPERTIES OF THE FERRICHROMES AND FERRITRIBENZOHYDROXAMATE AS A FUNCTION OF pH

	pH	λ_{max}	ϵ_{max}
Ferrichrome A	8	440	3740
	1.6	440	3460
	0 (1 N HCl)	445	1900
Ferrichrome	8	425	2895
	2.1	425	2790
	1.0	435	1970
	0 (1 N HCl)	470	425
Ferri-tribenzohydroxamate[a]	8	440	4050
	3.5	480	2860
	1.0	510	1450

[a] R. V. Christian, I. D. Leffler and J. S. Dahler, *Anal. Chem.*, **26**, 1666 (1954).

Acethydroxamic acid behaved similarly to the benzohydroxamic acid. These data indicate the greater stability of the ferrichrome complexes.

Identification of the Acyl Moieties of the Hydroxamic Acid Functions. Ferrichrome.—A 9.0 ml. aliquot of an aqueous solution of iron-free ferrichrome containing 2.52 μmoles per ml. was titrated electrometrically.[12] Exactly 75 μmoles of base were consumed with pK_a' of 9.1, corresponding to 3.3 moles per mole ferrichrome. The pH was readjusted to 7 with dilute HCl and 1.0 ml. of 0.10 N periodic acid was added. The solution was stirred for about one minute, 2 drops of ethylene glycol were added and the solution then retitrated. The buffer zone at pH 9.1 was now replaced completely by a new equivalent buffer zone with pK_a' of 4.7. From these data it is evident that periodate attacks the hydroxamate function and results in the liberation of three moles of the acyl group as the free acid. Control experiments with acethydroxamic acid supported this conclusion.

The acidified aqueous solution was extracted with ether in order to obtain the organic acid (extracted only with difficulty). The product was identified as acetic acid in the following manner: paper chromatography of the ammonium salt[30] gave only one spot corresponding to ammonium acetate. No non-volatile acids were present. The pK of 4.7 agrees with that of acetic acid. Finally, the ether extracts were concentrated to a small volume through a micro-distillation column and the residue analyzed by gas phase chromatography by the method of James and Martin.[31] A four foot Celite column, impregnated with stearic acid, was used at 100° with N_2 flow rate of 72 ml./min.

Organic acid	Retention volume
Acetic (standard)	0.677
Acid from ferrichrome	0.677
Propionic (control)	1.348

(26) T. Z. Csaky, *Acta Chem. Scand.*, **2**, 450 (1948). We have found some indication that the Csáky test may be given by alkyl hydroxylamines as well as by free NH_2OH.

(27) A. H. Blatt, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 69.

(28) J. M. Bremner, *Analyst*, **79**, 198 (1954).

(29) G. A. Snow, *J. Chem. Soc.*, 2588 (1954). The NH_2OH detected on these paper chromatograms may represent only a small fraction of the theoretical amount present in the original molecules.

(30) E. P. Kennedy and H. A. Barker, *Anal. Chem.*, **23**, 1033 (1951).

(31) A. T. James and A. J. P. Martin, *Biochem. J.*, **50**, 679 (1952).

Ferrichrome A.—The experiment was performed exactly as above except that 5.0 ml. of a solution containing 2.86 μmoles per ml. of iron-free ferrichrome A was used. Electrometric titration revealed 3.1 equivalents titrating with pK_a' of 4.0 and 3.1 equivalents titrating with pK of 9.3. The intact iron complex exhibits only the former buffer zone. After reaction with periodate the titration at pH 9.3 was eliminated and a new titration having the appearance of a dicarboxylic acid appeared with mid-point at pH 4.2.

Isolation of the Acid Moiety of Ferrichrome A.—To 6.5 ml. of a solution containing 148 mg. of iron-free ferrichrome A was added slowly 6.6 ml. of 0.10 N periodic acid. The acidic solution was extracted five times with 5 ml. portions of ether. The combined ether extracts were washed three times with 0.1 N HCl. The ether was evaporated under a stream of warm air and the residue dried overnight over $CaCl_2$. The product (36 mg.) was a white crystalline solid, m.p. 131–134°, very soluble in water, ether and alcohol, insoluble in benzene and petroleum ether Paper chromatography with 1-butanol:formic acid:water::100:15:150 revealed only one spot with R_f 0.87 (spray of 0.04% ethanolic chlor phenol red, pH 7). Electrometric titration gave a neutral equivalent of 72.

Anal. Calcd. for $C_6H_8O_4$: C, 49.99; H, 5.59. Found: C, 49.34; H, 5.34.

Baer test for unsaturation was positive. The ultraviolet absorption spectrum showed a peak at 217 mμ with ϵ_{max} of 10,600. Approximately 10 mg. was dissolved in 2 ml. of water, 0.2 ml. of concd. HCl and 10 mg. of palladium–charcoal catalyst were added, and a slow stream of H_2 then was passed through the solution until the 217 peak had disappeared. The acidic solution was extracted several times with ether. The residue obtained by evaporation of the combined ether extracts was taken up in the minimum volume of ether and crystallized by the addition of benzene. The melting point of the white crystalline solid was 82–84° and showed no depression on admixture with authentic β-methylglutaric acid (m.p. 84–85°, recryst. from benzene) but showed a 20 degree depression with α-methylglutaric acid (m.p. 75.5–77°). The infrared spectrum was identical with that of β-methylglutaric acid (1% KBr pellet).

A sample of pure *trans*-β-methylglutaconic acid, m.p. 130–135°, showed no melting point depression when mixed with our unsaturated acid. The two acids ran as a single spot when chromatographed on paper with a variety of solvents. The authentic acid showed identical ultraviolet and infrared spectra. Further confirmation was obtained by gas phase chromatography. Approximately 1 mg. of the acid was dissolved in 0.05 ml. of ether at 0°. A 5% excess of an ethereal solution of diazomethane, prepared by the method of McKay,[32] was added. After ten minutes, 0.01 ml. of the solution was examined with an Aerograph Model A-110-C Gas Chromatography apparatus at 193° with a helium flow rate of 50 ml./min. on a Craig succinate polyester column. Samples of the pure *cis*- and *trans*-β-methylglutaconic acids were similarly esterified and chromatographed.

Organic acid (di-methyl ester)	Retention volume
cis-β-Methylglutaconic acid	0.205
trans-β-Methylglutaconic acid	.265
Acid from ferrichrome A	.265

The acid content of ferrichrome A was determined quantitatively by the following procedure: 0.50 ml. of a solution containing 2.13 μmoles of iron-free ferrichrome A was treated with periodate as above. One drop of ethylene glycol was added and the solution extracted five times with ether. The ether was evaporated carefully and the crystalline residue quantitatively transferred to a volumetric flask and made up to the mark with 0.01 N HCl. The optical density of this solution at 217 mμ was determined with a Beckman DU spectrophotometer. The amount of acid present was calculated by reference to a standard curve of the authentic acid. Controls showed no iodate carried over during the extraction and the ratio of optical density at 217 mμ to 240 mμ agreed with that of the authentic acid. Duplicate analyses showed 3.1 moles of β-methylglutaconic acid per mole of ferrichrome A.

Acknowledgments.—The authors are indebted to James Cason, J. D. Cawley, Keith Freeman, D. M. Greenberg and R. H. Wiley for gifts of chemicals or advice.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF NORTHWESTERN UNIVERSITY]

3-Bromo-2,2-dimethylcoumaran and its Reactions

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RECEIVED OCTOBER 31, 1959

The reaction of 2,2-dimethylcoumaran with N-bromosuccinimide yielded a highly reactive 3-bromo-2,2-dimethylcoumaran whose structure was proved by its conversion, *via* the acetate, the alcohol and the ketone, to a bright-red 2,4-dinitrophenylhydrazone, and through its reaction with methylmagnesium iodide which gave the known 2,2,3-trimethylcoumaran. While with zinc in refluxing benzene the bromide gave a polycoumaran, with magnesium or with zinc in ether solution it gave two compounds believed to be stereoisomers of 2,2,2',2'-tetramethyl-3,3'-bicoumaran. With sodium in ether solution 3-bromo-2,2-dimethylcoumaran gave a low yield of 2-isobutenylphenol. Grignard reagents and 3-bromo-2,2-dimethylcoumaran gave mainly 3-alkylated coumarans, which furnishes a convenient method of preparing 2,2,3-trialkylcoumarans from 2,2-dialkylcoumarans.

In continuation of the studies[2] of the cleavage of O-heterocyclic compounds, such as coumarans, chromans and benzofurans, the preparation of a model compound 3-bromo-2,2-dimethylcoumaran and its ring-opening reactions were studied because of intrinsic interest and also in connection with the possibility of application in the degradation of lignin. The results obtained, although hardly suitable to the application in the degradation of coumaran-containing materials, such as lignin, seem worth reporting on their own merit.

The reaction of N-bromosuccinimide[3] with coumarans has been described on several occasions,[4] but the isolation of the resulting bromocoumarans has not been reported. One such compound, 3-bromo-2,2-dimethylcoumaran (II), has been isolated and characterized in the present work.

An attempt to obtain a bromide from 2-methylcoumaran and N-bromosuccinimide in carbon tetrachloride failed because of the almost explosive violence of the reaction and dehydrobromination of the resulting reaction mixture. Although conditions more favorable to the desired course of the reaction and isolation might have been found, this was not

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