

Chlorination of Stilbene.—In the absence of added peroxide, stilbene reacts slowly with sulfuryl chloride. Whether this slow reaction is due to a trace of peroxide, or whether it is a slow thermal bimolecular reaction, could not be determined. The addition of a small quantity of benzaldehyde or ascaridole brings about a rapid, vigorous reaction at room temperature. The yield of crude product (stilbene dichloride) is quantitative. After fractional crystallization of this crude material, first from alcohol to obtain the higher-melting isomer, and then from ligroin to obtain the other isomer, there was obtained a 45% yield of α, α' -stilbene dichloride, m. p. 191–193°, and a 33% yield of the β, β' isomer, m. p. 90–93°.

Chlorination of Tetraphenylethylene.—Tetraphenylethylene, like stilbene, reacts slowly with sulfuryl chloride in the absence of added peroxide. When a trace of a peroxide (benzaldehyde which

gives a strong peroxide test, or ascaridole) is added, the reaction is accelerated tremendously. The product formed is the dichloride, m. p. 184–186° (dec.).

Summary

1. Sulfuryl chloride reacts with ethylenic compounds to form the corresponding dichlorides. The following olefins have been chlorinated: cyclohexene, allyl chloride, dichloroethylene, tetrachloroethylene, stilbene, and tetraphenylethylene.

2. The chlorination of these ethylenic compounds by means of sulfuryl chloride is catalyzed by organic peroxides.

3. It is suggested that the reaction proceeds through a chain mechanism involving atoms and free radicals.

CHICAGO, ILLINOIS

RECEIVED OCTOBER 11, 1939

[CONTRIBUTION FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY AND THE WILLIAM G. KERCKHOFF LABORATORIES OF THE BIOLOGICAL SCIENCES, CALIFORNIA INSTITUTE OF TECHNOLOGY]

The Wound Hormones of Plants. IV. Structure and Synthesis of a Traumatin¹

BY JAMES ENGLISH, JR.,^{1a} JAMES BONNER AND A. J. HAAGEN-SMIT

Many plant tissue extracts are capable of inducing renewed growth activity in mature plant cells. These growth inducing extracts are usually obtained from ground or heated tissues, and they are capable of evoking cell division and cell enlargement in unwounded cells. The active principles have therefore been referred to as "wound hormones."² The present paper concerns the structure and synthesis of one highly active "wound hormone," 1-decene-1,10-dicarboxylic acid, whose isolation has already been announced.³ The authors propose that the name "traumatic acid" be used in referring to this substance.

In earlier papers⁴ a quantitative bio-assay (the bean test) for wound hormone activity has been described.

Activity tests of the material described below

(1) This paper describes work begun under a grant from the Lalor Foundation. Report of work carried out with the cooperation of the Works Progress Administration, Official Project Number 665-07-3-83, Work Project Number 9809.

(1a) Present address: Yale University, New Haven, Conn.

(2) (a) G. Haberlandt, *Beil. z. allgemeinen Botanik*, **2**, 1 (1921); (b) G. Haberlandt, *Biol. Zentr.*, **42**, 145 (1922).

(3) J. English, J. Bonner and A. Haagen-Smit, *Proc. Nat. Acad. Sci.*, **25**, 323 (1939).

(4) J. English and J. Bonner, *Jour. Biol. Chem.*, **121**, 791 (1937); J. Bonner and J. English, *Plant Physiol.*, **13**, 331 (1938).

were always carried out in the presence of a "co-factor"⁵ mixture.

One-tenth gamma of the pure substance per applied drop elicits a detectable response in the bean test. The pure product, m. p. 165–166°, is optically inactive and yielded an equivalent weight by titration of 118. A micro-Zerewitinoff determination carried out on the dimethyl ester showed the absence of active hydrogen. Catalytic hydrogenation yielded decane-1,10-dicarboxylic acid, identified by melting point and mixed melting point with an authentic sample prepared from decamethylene glycol.⁶

Since the analysis and the result of the hydrogenation experiment indicated the existence of one double bond, an oxidative degradation was carried out. On oxidation of the methyl ester with permanganate in acetone solution, sebacic acid was formed indicating the presence of a double bond in the α - β position. This conclusion was confirmed by the synthesis of 1-decene-1,10-di-

(5) For a description of the preparation and properties of this co-factor mixture, see note 3. Sucrose and particularly glutamic acid can in part replace the co-factor mixture in augmenting the activity of traumatic acid.

(6) P. Chuit, *Helv. Chim. Acta*, **9**, 264 (1926).

carboxylic acid which proved to be identical with the natural active material. The synthesis was carried out by converting undecylenic acid to the half aldehyde of sebacic acid⁷ and condensing this with malonic acid in the presence of pyridine. Carbon dioxide was split off as expected⁸ and the unsaturated acid was isolated after hydrolysis of the ester group.

During the course of the investigation a large number of pure organic substances was tested (in the presence of the co-factor mixture noted above) for activity in the bean test. With the exception of certain homologs and analogs of decene-dicarboxylic acid, these materials (indole-acetic acid, vitamins B₁, B₂, B₆, and other known plant growth substances as well as numerous organic acids) were wholly without activity. The hydrogenation product, decane-1,10-dicarboxylic acid, possesses an activity approximately half that of decene-1,10-dicarboxylic acid. The presence of the double bond, therefore, while not essential, nevertheless enhances the activity of this molecule. The saturated dibasic acids possessing 7 or fewer C atoms were without significant activity. Suberic and azelaic acids with 8 and 9 C atoms, respectively, possess slight activity and sebacic acid (10 C atoms) is approximately half as active as decene-1,10-dicarboxylic acid. It is of interest that although succinic acid is completely inactive, maleic acid evokes a small but definite response in the bean test. In this case, as in the case of the C₁₂ acids, the presence of one double bond increases wound hormone activity. Lauric acid, with 12 C atoms and but one carboxyl group is completely inactive as are the unsaturated monobasic acids linolic and undecylenic. Two carboxyl groups would therefore appear to be essential for wound hormone activity in the bean test.

The physiological activities of decene-dicarboxylic acid and the related higher dicarboxylic acids are not restricted to the evoking of intumescences in bean pods. Synthetic decene-dicarboxylic acid is capable of evoking intense cell division activity in the "potato test" of Haberlandt.⁹ The activity of decene-dicarboxylic acid in this test is further enhanced by the co-factor mixture described above in connection with the

bean test. It seems probable therefore that traumatic acid is one of the substances responsible for wound periderm formation in the potato.

Synthetic decene-dicarboxylic acid is also capable of partially inhibiting the germination of seeds, particularly those seeds which are borne in fleshy fruits such as tomato, and it seems possible therefore that traumatic acid is in nature partly responsible for the failure of such seeds to germinate while they remain within the fruit. A fuller account of these physiological investigations will be published elsewhere.

Experimental

Isolation Procedure.—The preparation of the crystalline active material was carried out as described in a previous paper with the added precaution that all solvent evaporations were carried out below 35°. In this way there was obtained, after repeated recrystallization from alcohol, 56.6 mg. of pure material, m. p. 165–166°; 40 mg. dissolved in 2 cc. of absolute alcohol showed no measurable optical rotation in a 10-cm. tube.

Hydrogenation.—Nine milligrams of the product was hydrogenated by shaking with hydrogen at atmospheric pressure in glacial acetic acid in the presence of platinum oxide. On filtering the catalyst and removing the solvent, there was obtained a crystalline product which, after one recrystallization from alcohol, melted at 127° and was pure decene-1,10-dicarboxylic acid. Mixture with an authentic sample showed no m. p. depression.

Five milligrams of the pure reduction product was converted to the anilide by heating with thionyl chloride, removing the excess reagent *in vacuo* and adding aniline. After heating on the steam-bath for fifteen minutes the product was worked up in the usual manner. After recrystallization from alcohol, it gave a melting point of 171° and showed no melting point depression with a sample prepared from synthetic decane-1,10-dicarboxylic acid.

*Anal.*¹⁰ Calcd. for C₂₄H₃₂O₂N₂: N, 7.38. Found: N, 7.38, 7.28.

Oxidation.—Eighteen milligrams of the pure crystalline material was dissolved in ether and an ether solution containing an excess of diazomethane was added. The crude methyl ester⁴ obtained on evaporation of the ether was dissolved in acetone and oxidized by adding an acetone solution of potassium permanganate at room temperature until an excess was present. The solution was then refluxed and permanganate added until no more was taken up. The acetone was removed under reduced pressure from the filtered solution and the residue was dissolved in 4 normal sodium hydroxide. This solution was then heated on the steam-bath for two hours, cooled, acidified and extracted with ether. Upon evaporation of the ether there was obtained 14 mg. of crude acid which, after recrystallizing twice from alcohol, melted at 133° and was found to be identical with sebacic acid by mixed melting point. This product yielded an amide, m. p. 200°, identical with sebacic acid di-amide.

(7) C. R. Noller and R. Adams, *THIS JOURNAL*, **48**, 1074 (1926).

(8) W. J. Hickinbottom, "Reactions of Organic Compounds," Longmans, Green and Co., New York, N. Y., p. 147.

(9) G. Haberlandt, *Sitzber. Kgl. preuss. Akad. Wiss.*, **16**, 318 (1918).

(10) Microanalysis by G. A. Swinehart.

Synthesis of 1-Decene-1,10-dicarboxylic Acid.—Undecylenic acid methyl ester (b. p. 119–120°, 10 mm.) prepared from Eastman Kodak Co. undecylenic acid was ozonized according to the procedure of Noller and Adams⁷ in acetic acid solution and the half aldehyde of methyl sebacate (b. p. 124–125° (4 mm.)) obtained in 40% yield. Thirteen grams of this product was mixed with 15 g. of malonic acid and 15 g. of dry pyridine and heated under reflux for four hours. A vigorous evolution of carbon dioxide was observed in the early stages of the reaction. The resulting clear solution was acidified, poured into water and extracted with ether. After removal of the pyridine by extraction with dilute hydrochloric acid, the ether was evaporated and the crude product hydrolyzed directly by heating under reflux with 3 normal sodium hydroxide for two hours. Upon acidification the 1-decene-1,10-dicarboxylic acid precipitated and was recrystallized from alcohol or acetone, m. p. 165–166°. This product showed no depression of melting point when mixed with the naturally occurring material and was identical with the latter in crystalline form and physiological activity.

Anal. Calcd. for C₁₂H₂₀O₄: C, 63.05; H, 8.80. Found: C, 62.98; H, 8.85.

The S-benzyl thiuronium salt of traumatic acid was prepared according to the method of Donleavy.¹¹

Anal. Calcd. for C₂₈H₄₀O₄N₄S₂: C, 59.96; H, 7.79; N, 9.99. Found: C, 59.78; H, 7.49; N, 9.90.

(11) J. J. Donleavy, *THIS JOURNAL*, **58**, 1004 (1938).

An oxidation of the synthetic product with permanganate in hot water solution gave sebacic acid, identified by melting point (133°) and mixed melting point.

Summary

The crystalline substance isolated from string bean pods and capable of inducing renewed cell division and cell extension activity in the parenchymatous cells of the bean pod mesocarp is shown to be 1-decene-1,10-dicarboxylic acid. Synthetic 1-decene-1,10-dicarboxylic acid was prepared and shown to be identical with the natural product both in physical properties and in physiological activity. It is proposed that the name "traumatic acid" be used in referring to this substance.

Traumatic acid is also shown to be capable of inducing wound periderm formation in washed disks of potato tuber and to function thus as a wound hormone of the potato. Traumatic acid is also capable of partially replacing the juice of the tomato fruit in reversibly inhibiting the germination of tomato seeds.

PASADENA, CALIF.

RECEIVED AUGUST 10, 1939

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

The System *n*-Butylmagnesium Bromide–Magnesium Bromide–Ethyl Ether

BY W. E. DOERING AND C. R. NOLLER

The system magnesium bromide–ethyl ether has been investigated by Menschutkin¹ who found that the solubility of the dietherate varied from 1.08% at –8° to 5.80% at 22°. At 22.8° the dietherate melted under ether to give two liquid phases. The composition of the upper layer changed from 6.8% dietherate at 30° to 10.2% at 90° while that of the lower layer changed from 69.8% at 30° to 68.6% at 90°.

During the course of another investigation² it was found that quantities of magnesium bromide greatly in excess of its solubility in ether could be added to Grignard solutions without the separation of two phases and in connection with studies on the composition and constitution of Grignard reagents it became desirable to determine quantitatively the effect of Grignard reagents on the solubility of magnesium halides. Table I and Fig. 1 summarize our results on the

system *n*-butylmagnesium bromide–magnesium bromide–ethyl ether. Since the molecules of both the Grignard reagent and the magnesium bromide are present in all probability as the dietherates at ordinary temperatures when an excess of ether is present, the numerical values have been calculated on this basis. It is realized of course that the system is more complicated than indicated because of the equilibrium between *n*-butylmagnesium bromide, di-*n*-butylmagnesium and magnesium bromide. Since it is not possible at the present time to determine accurately the position of this equilibrium,³ all of the active Grignard reagent has been calculated as being in the form of *n*-butylmagnesium bromide.

It can be seen that the presence of *n*-butylmagnesium bromide greatly modifies the solubility of magnesium bromide dietherate. Whereas magnesium bromide dietherate and ether form homogeneous solutions at 25° only when the con-

(1) Menschutkin, *Z. anorg. Chem.*, **49**, 34, 207 (1908).

(2) Probert, *Master's Thesis, Stanford University, 1938.*

(3) Noller and White, *THIS JOURNAL*, **59**, 1354 (1937).