

carbonate solution, then with water and dissolved in ether. The ether solution was dried with fused sodium sulfate and the solvent removed. All the esters were oils and were obtained in about 10 g. yields.

**1-Alkyl 2-( $\beta$ -Diethylaminoethyl) 4-Nitrophthalate (III).**

—(a) One-hundredth mole of the 1-alkyl 4-nitroacidphthalate, 0.01 mole of  $\beta$ -diethylaminoethyl chloride<sup>7</sup> and 30 cc. of isopropyl alcohol were heated for ten hours on a steam-bath. The solvent was removed under reduced pressure and the oily ester hydrochloride washed with anhydrous ether whereupon it usually solidified. The salt was recrystallized from a mixture of alcohol and ethyl acetate.

In the event that the hydrochloride could not be obtained in a crystalline state, the base was liberated and the hydrobromide or methiodide was prepared.

(b) A mixture of 0.056 mole of the 1-alkyl 2-( $\beta$ -bromoethyl) 4-nitrophthalate, 20 cc. of dry toluene and 16.5 g. of diethylamine was heated in a magnesium citrate bottle at 95° in a water-bath for three hours. After filtration of the precipitated diethylamine hydrobromide, the solvent and the excess amine were removed under reduced pressure. The residue was washed with water, dissolved in 5% hydrochloric acid, the mixture filtered, the filtrate made alkaline with 10% sodium carbonate solution and the ester

(7) Slotta and Behnisch, *Ber.*, **68**, 758 (1935).

extracted with ether. After the ether extract had been dried with fused sodium sulfate, the solvent was removed and the oily, basic ester was rubbed with the calcd. amount of 38% hydrochloric acid required for the formation of the hydrochloride. The product became crystalline after it had been washed thoroughly with dry ether.

Esters which contained the 2-( $\beta$ -dipropylaminoethyl), 2-( $\beta$ -piperidinoethyl) or 2- $\beta$ -(4-morpholy)-ethyl group were prepared by substitution of dipropylamine, piperidine or morpholine for diethylamine in the above procedure. The hydrochlorides of these esters were obtained by the addition of the required amount of alcoholic hydrogen chloride to the base.

**1-Alkyl 2-( $\beta$ -Diethylaminoethyl) 4-Aminophthalate (IV).**

—All of the nitro esters listed in Table I were reduced to the corresponding amino compounds with stannous chloride and hydrogen chloride in acetic acid solution.<sup>8</sup>

### Summary

A number of 1-alkyl 2-dialkylaminoalkyl 4-aminophthalates have been described. Most of them exhibit a fair degree of local anesthetic activity when tested on the rabbit cornea.

(8) Blicke and Parke, *This Journal*, **61**, 1201 (1939).

ANN ARBOR, MICHIGAN

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[CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS]

## Structure of Gossypol. XXVI. Gossypolic Acid<sup>1</sup>

BY ROGER ADAMS, T. A. GEISSMAN, W. R. DIAL AND J. T. FITZPATRICK<sup>2</sup>

With one exception, all of the significant degradation products of gossypol have been discussed in previous papers and their structures explained satisfactorily on the basis of the postulated formula for gossypol (I).<sup>3</sup> The exception is gossypolic acid, reported by Karrer and Tobler<sup>4</sup> to be formed by ozonization of gossypol along with oxalic acid. Unfortunately the conditions for preparation and isolation of gossypolic acid were not well established and the inability of these authors to obtain more than very small amounts of material prevented them from making an exhaustive study of this substance. They suggested, however, that it was probably an aromatic *o*-hydroxy acid as determined by its solubility in aqueous sodium bicarbonate and the color reaction with alcoholic ferric chloride. On the basis of

(1) For previous paper in this series, see Adams and Baker, *This Journal*, **63**, 535 (1941).

(2) An abstract of a thesis submitted in partial fulfillment for the degree of Doctor of Philosophy in Chemistry; Solvay Process Co. fellow 1940-1941.

(3) Adams, Morris, Geissman, Butterbaugh and Kirkpatrick. *This Journal*, **60**, 2193 (1938).

(4) Karrer and Tobler, *Helv. Chim. Acta*, **15**, 1204 (1932).

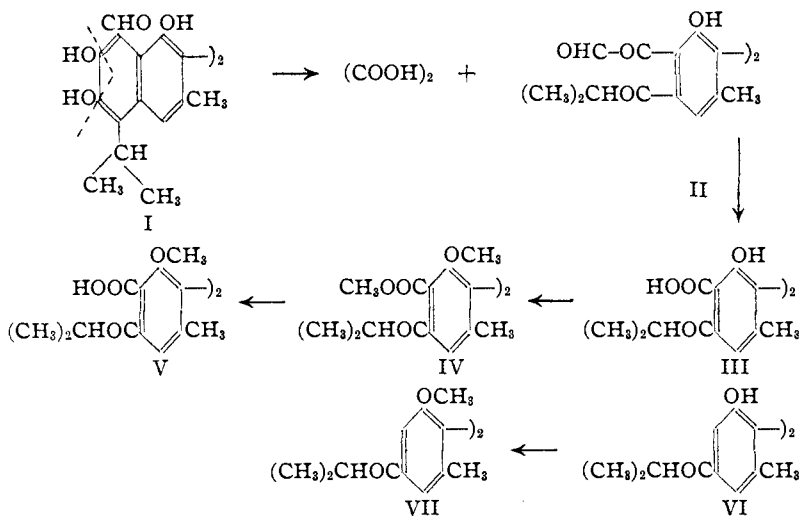
analytical data, it was assigned the formula (C<sub>12</sub>H<sub>14</sub>O<sub>4</sub>)<sub>x</sub>. Molecular weight determinations gave values half-way between those expected, if  $x = 1$  and  $x = 2$ .

Gossypolic acid with diazomethane was converted into a molecule with two methoxyl groups per unit (C<sub>11</sub>H<sub>12</sub>O·OCH<sub>3</sub>·COOCH<sub>3</sub>)<sub>x</sub>, one of which was saponified readily by alkali to give an aqueous bicarbonate soluble product (C<sub>11</sub>H<sub>12</sub>O·OCH<sub>3</sub>·COOH)<sub>x</sub>. The molecular weight of the methyl ether methyl ester gave a value between 10 and 15% too low for a substance in which  $x = 2$ .

After many experiments, it was found possible in this investigation to obtain a small amount of gossypolic acid. Even the successful ozonolyses under carefully observed conditions could not be duplicated more than occasionally. As a consequence, the experiments have been limited in scope. The reactions of Karrer and Tobler on gossypolic acid were repeated in all details. Complete analyses on the three products and a molecular weight determination on the methyl ether methyl ester were performed. From the results,

the formula of gossypolic acid is proposed as  $(C_{12}H_{13}O_4)_2$ . It is to be noted that thirteen hydrogens are suggested in place of the fourteen in Karrer and Tobler's formula. The analytical data admittedly are not sufficiently accurate to distinguish with certainty between thirteen and fourteen hydrogens but agree a little better with the former value and are more consistent with a formulation derived from the structure of gossypol. The molecular weight determination of the ether ester warrants an acceptance of two units in the molecule (calcd., 498; found, 485).

Various ways were postulated in which gossypol (I) could be ozonized to give a phenolic acid of formula  $(C_{12}H_{13}O_4)_2$  and oxalic acid. Only one of these appears entirely plausible and is shown in



structures I, II and III of which II is a possible hypothetical intermediate. Structure III is, therefore, suggested as that of gossypolic acid and structures IV and V for its derivatives previously discussed.

The quantity of gossypolic acid available was sufficient to attempt a decarboxylation by heating with quinoline and copper. A product was thus obtained the analytical data for which agree with those of structure VI. Upon methylation, a dimethyl ether resulted presumably with structure VII. All these products (III-VII) are biphenyl derivatives and it should be possible to synthesize at least the compound VI or VII by which the correctness of these formulas could be established.

The proof that the ozonization degradation product of gossypol is a derivative of 2,2'-dihydroxy-6,6'-dimethylbiphenyl would be of more

than minor significance. Numerous attempts to oxidize gossypol or its derivatives in such a way as to eliminate the outer benzene rings of the binaphthyl nuclei to give a biphenyl derivative have all failed. Perhaps ozone has resulted in an oxidation in this direction. Of the complex gossypol molecule, the only part which has not been established by direct chemical evidence is the relative positions of the methyl groups and the link between the two naphthalene nuclei. The identification of compound VI or VII as a 2,2'-dihydroxy-6,6'-dimethylbiphenyl derivative would, therefore, not merely clarify the structure of gossypolic acid but would complete the determination of the structure of gossypol beyond any possible contradiction.

### Experimental

**Gossypolic Acid (III).**—A solution of 20 g. of twice recrystallized gossypol-acetic acid in 3 kg. of glacial acetic acid was cooled to room temperature and ozone (about 2-3%) passed in. The solution, maroon color at first, became during a period of about three hours red, then yellow and finally after eighteen to twenty-four hours greenish-yellow. The reaction was stopped at this point since no further change was observed. This is in contrast to the results of Karrer and Tobler,<sup>3</sup> who reported ozonization for nine days with eventual formation of a blue color. A colorless solution in ozonizations performed in this Laboratory was never obtained, regardless of the time of ozonization.

The solvent was removed under diminished pressure and, when the volume had reached 100 cc., the solution was diluted with a liter of water. After a few hours of standing, the precipitate was filtered. It weighed 2-3 g. The purification was troublesome. The crude product was dissolved in aqueous sodium bicarbonate, treated with Norite and reprecipitated with hydrochloric acid. The material was then crystallized from benzene and finally from dilute ethanol or dilute acetic acid. The yield seldom exceeded 0.5 g. It formed a white crystalline powder, m. p. 241° (cor.). Karrer and Tobler reported m. p. 241° (uncor.).

*Anal.* Calcd. for  $(C_{12}H_{13}O_4)_2$ : C, 65.15; H, 5.92. Found: C, 65.11; H, 5.95.

Unfortunately the procedure described above was successful only in an occasional experiment. Frequently the crude product was of oily consistency from which no solid gossypolic acid could be extracted.

A variety of other conditions was attempted: time of ozonization from six to forty-eight hours; hydrogen per-

oxide to decompose the ozonide; as solvents, acetone, benzene, dioxane, carbon tetrachloride; ozone up to 6% concentration; temperatures from 0–30° in carbon tetrachloride and in dioxane; replacement of gossypol by gossypol hexamethyl ether and anhydrogossypol. None was successful.

**Dimethyl Gossypolate Dimethyl Ether (IV).**—A solution of 2 g. of gossypolic acid in 40 cc. of dry ether was cooled and added to a dry ether solution of diazomethane (from 3 g. of nitrosomethylurea). The mixture was allowed to stand overnight at 0–5°, the excess diazomethane then destroyed with acetic acid and the solution washed with aqueous sodium bicarbonate. Upon evaporation of the ether an oil remained which was dissolved in 10–12 cc. of ethanol. Upon cooling, white crystals separated which were recrystallized from dilute methanol; flat white needles, m. p. 138–139° (cor.). Karrer and Tobler reported m. p. 142° (uncor.).

*Anal.* Calcd. for  $C_{23}H_{34}O_5$ : C, 67.47; H, 6.83; mol. wt., 498. Found: C, 67.39; H, 6.83; mol. wt. (camphor), 485.

**Gossypolic Acid Dimethyl Ether (V).**—A solution of 0.92 g. of dimethyl gossypolate dimethyl ether in 3 cc. of 10% methanolic potassium hydroxide was allowed to stand for three days at room temperature. The solvent was removed under diminished pressure and the residue dissolved in water. After filtering the aqueous solution, it was acidified with dilute sulfuric acid. After two recrystallizations from dilute ethanol it formed tiny white prisms, m. p. 231–233° (cor.). Karrer and Tobler reported m. p. 225° (uncor.).

*Anal.* Calcd. for  $C_{26}H_{30}O_5$ : C, 66.35; H, 6.42. Found: C, 66.18; H, 6.57.

**Decarboxylated Gossypolic Acid (VI).**—A mixture of 1 g. of gossypolic acid, 3 cc. of quinoline and 1 g. of copper powder was heated to 180–185° under an atmosphere of nitrogen for one hour. It was then allowed to cool and about 50 cc. of ether was added. The solution was filtered and then washed successively four times with 5% hydrochloric acid, once with water, twice with 5% aqueous sodium bicarbonate and again with water. After drying the ether solution and evaporating the solvent, the residue was washed with 10 cc. of dry ether which removed most of the tar and left a brown solid. This was dissolved in about 15 cc. of methanol and the solution allowed to evaporate to about 3 cc. The liquid was then decanted and the remaining solid washed with 5 cc. of dry ether and redissolved in 10 cc. of methanol. The above process was repeated twice. Finally the product was dissolved in 15 cc. of dry ether and the solution allowed to evaporate to 3 cc. The very light tan crystalline solid left after decanting the

solvent had a m. p. 252–253° (cor.); yield of purified product 0.12 g. (15%). This product gives no color with ethanolic ferric chloride.

*Anal.* Calcd. for  $C_{22}H_{26}O_4$ : C, 74.55; H, 7.39. Found: C, 74.20; H, 7.78.

Some unchanged gossypolic acid could be recovered from the original aqueous bicarbonate wash liquors.

**Decarboxylated Gossypolic Acid Dimethyl Ether (VII).**—A mixture of 30 mg. of pure decarboxylated gossypolic acid and 1 cc. of dimethyl sulfate was heated until all the solid had dissolved. A concentrated solution of potassium hydroxide was added dropwise until the solution became homogeneous. The rate of addition was such as to keep the solution just below the boiling point. The mixture was then refluxed for five minutes and allowed to cool to room temperature. Upon addition of water to a volume of 50 cc., a tarry product separated which solidified on standing. The solution was filtered and the solid dissolved in 25 cc. of methanol. Upon evaporation, an oily residue resulted which solidified on standing. After boiling for ten minutes with aqueous 10% potassium hydroxide, the hot solution was filtered and the undissolved material recrystallized from a mixture of dioxane and water. Five recrystallizations resulted in white crystals of constant m. p. 125–127° (cor.); yield 10 mg. (30%).

*Anal.* Calcd. for  $C_{24}H_{30}O_4$ : C, 75.36; H, 7.91;  $CH_3O$ , 16.2. Found: C, 75.58; H, 7.96;  $CH_3O$ , 15.2.

### Summary

1. Gossypolic acid was prepared by the ozonization of gossypol. Its molecular formula is suggested as  $(C_{12}H_{13}O_4)_2$  and its properties indicate it to be an aromatic *o*-hydroxy acid.

2. On the basis of the structure of gossypol, it is suggested that gossypolic acid probably is a biphenyl derivative. Heating with quinoline and copper causes loss of two carboxyl groups. The substance thus formed would then be 2,2'-dihydroxy-6,6'-dimethyl-4,4'-diisobutyrobiphenyl. It was methylated to its dimethyl ether. Establishment of the structure of this compound or its ether by synthesis would not merely prove the formula assigned to gossypolic acid but also would give direct and conclusive evidence for the proposed relative positions of the methyl group and the linkage between the naphthalene nuclei in the gossypol molecule.

URBANA, ILLINOIS

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