heated with naphthoquinone at 50°, a result hardly to be expected.

In contact with maleic anhydride at 30°, the hydrocarbon products gave a crystalline compound which was different from the adduct obtained by Farmer and Warren[3] from hexatriene and maleic anhydride at 100°. Since Farmer and Warren considered their compound to be an ethylidenetetrahydrophthalic anhydride, it appeared likely that the new isomer was the vinyltetrahydrophthalic anhydride, the normal adduct, and this view was expressed publicly.[4]

This has now been shown not to be the case. The substance, m. p. 147°, does not depress the m. p. of the ethanotetrahydrophthalic anhydride prepared[5] from cyclohexadiene and maleic anhydride. This is further evidence that the hydrocarbon obtained by the dehydration of the hexadienol contained cyclohexadiene. While the possibility of a direct hexatriene → cyclohexadiene transformation is still not excluded, it must be supposed that it occurs, if at all, during the dehydration of the hexadienol rather than during the reaction with naphthoquinone or maleic anhydride at 50 or 30°. However, it appears that the hexatriene prepared by this method is not always contaminated with so much cyclohexadiene, and the formation of the latter must depend on small variations in procedure, because the hydrocarbon has been found[6] not to yield, upon reaction with 5-acetoxy-1,4-toluquinone, any of the adducts obtained from cyclohexadiene and this quinone.

(3) Farmer and Warren, *J. Chem. Soc.*, 897 (1929).
(4) *J. Wash. Acad. Sci.*, **29**, 548 (1939).
(5) I. G. Farbenindustrie A.-G., *Chem. Zentr.*, **100**, II, 2502 (1929).
(6) E. Butz and L. Butz, *J. Org. Chem.*, **7**, 199 (1942).

BUREAU OF ANIMAL INDUSTRY
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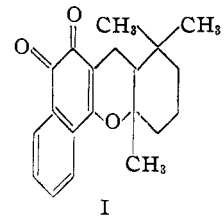
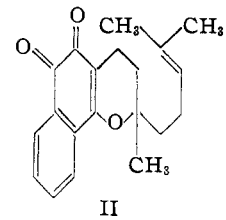
The Condensation of β-Cyclogeraniol with Leucoisonaphthazarin

BY MARSHALL D. GATES AND FERNANDA MISANI

In a recent paper Fieser and Gates[1] described the preparation of β-geranolapachone, a member of the β-lapachone series, by cyclization of 2-hydroxy-3-geranyl-1,4-naphthoquinone with concentrated sulfuric acid. The high melting point of this compound (234°) suggests that further cyclization of the side chain may have taken place to give structure I rather than the supposed structure II.

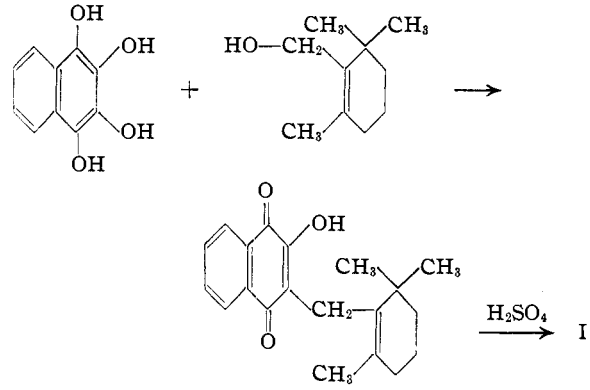


I



II

A choice between these two structures was readily made by synthesizing structure I by application of the general scheme employed by Fieser and Gates.[1]



The β-cyclogeraniol employed (m. p. 44°) was prepared by Meerwein–Ponndorf reduction of β-cyclocitral according to Kuhn and Hoffer.[2] Condensation with leucoisonaphthazarin gave the desired 2-hydroxy-3-(β-cyclogeranyl)-1,4-naphthoquinone in rather poor yield, but in easily isolated form. On cyclization with sulfuric acid, β-cyclogeranolapachone (I) was obtained. It proved to be identical with β-geranolapachone prepared according to Fieser and Gates, and their β-geranolapachone must therefore be regarded as β-cyclogeranolapachone (I).

The conditions used in the condensation of allylic alcohols with hydroxyhydroquinones in these syntheses are not sufficiently acidic (oxalic acid) to bring about cyclization of the geranyl group during the condensation. This is shown by the non-identity of the products obtained by the condensation of geraniol and β-cyclogeraniol with leucoisonaphthazarin.

Experimental Part[3]

Isonaphthazarin.—The following procedure represents an improvement over that reported by Fieser and Gates.[1] A solution of 8.9 g. of 2-hydroxy-1,4-naphthoquinone

(1) Fieser and Gates, THIS JOURNAL, **63**, 2948 (1941).
(2) Kuhn and Hoffer, *Ber.*, **67**, 357 (1934).
(3) All melting points are corrected.

(purified through the methoxy compound) in 500 cc. of water containing 4.3 g. of sodium bicarbonate was treated with 20 cc. of 30% hydrogen peroxide (superoxol). The solution rapidly darkened and, after standing for thirty-four hours, a crop of dull red large leaves had separated, 3.2 g. (43% based on 2-hydroxy-1,4-naphthoquinone utilized). One crystallization from dioxane gave pure isonaphthazarin. Acidification of the aqueous filtrate yielded 2.1 g. of orange precipitate which consisted of unchanged 2-hydroxy-1,4-naphthoquinone plus a small amount of isonaphthazarin. On several occasions, use of less pure 2-hydroxy-1,4-naphthoquinone as starting material led to much lower yields.

2-Hydroxy-3-(β-cyclogeranyl)-1,4-naphthoquinone.—Isonaphthazarin (2.0 g.) was reduced as described by Fieser and Gates[1] and the leuco compound heated in the dark under nitrogen for forty-eight hours at 65–70° with 1.0 g. of β-cyclogeraniol[2] (m. p. 44°), 0.6 g. of anhydrous oxalic acid and 20 cc. of dioxane. The processing of the reaction mixture included the following steps: extraction of the unchanged leucoisonaphthazarin with aqueous hydrosulfite, reduction with concentrated aqueous hydrosulfite, and extraction from ether–petroleum ether with Claisen's alkali. The crude phenolic portion thus obtained was chromatographed after air oxidation on freshly ignited magnesium sulfate. On development with petroleum ether, a weakly adsorbed bright yellow band readily passed into the filtrate. Similar filtrates from systematic readsorptions of the column eluate were combined and on concentration to dryness under reduced pressure afforded 99 mg. of solid residue which after three crystallizations from ether–petroleum ether gave 36 mg. of golden yellow rectangular plates, m. p. 135–135.5°. It is quite soluble in the ordinary organic solvents, fairly soluble in warm petroleum ether, much less soluble cold, and dissolves in dilute alcoholic alkali to give the beautiful scarlet characteristic of alkali salts of 2-hydroxy-1,4-naphthoquinones. It dissolves in concentrated sulfuric acid to give a deep orange-red solution. Two further crystallizations to obtain a sample for analysis did not alter the melting point.

Anal. Calcd. for $C_{20}H_{22}O_3$: C, 77.38; H, 7.15. Found: C, 77.50; H, 7.23.

β-Cyclogeranolapachone (I).—A solution of 11 mg. of 2-hydroxy-3-(β-cyclogeranyl)-1,4-naphthoquinone in ice-cold concentrated sulfuric acid (0.3 cc.) was allowed to stand several minutes, then diluted with ice water. The precipitated dark orange-brown material was taken into ether, washed with water, bicarbonate and brine, and concentrated to dryness. The residue was taken into benzene–hexane and chromatographed on freshly ignited magnesium sulfate. Development with 50% benzene–hexane left a broad salmon-pink band in the middle of the column which was sectioned out and eluted with ether. After evaporation of the ether, the solid residue was crystallized twice from pure acetone to give 3.5 mg. of orange-red prismatic blades, m. p. 232–233.3°. A mixed melting point with β-geranolapachone prepared according to Fieser and Gates[1] showed no depression.

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Riboflavin Estimation in Fruits and Vegetables

BY G. MACKINNEY AND J. M. SUGIHARA

As part of a collaborative project,[1] it was recently necessary to make a series of chemical determinations of thiamin and riboflavin in certain fruits and vegetables, and the Conner–Straub procedure[2] was followed. Unfortunately, at the beginning, Supersorb,[3] the specific adsorbent for riboflavin was unavailable. An empirical method was, therefore, evolved, and we hoped, later, to correlate results into the series by concurrent assays on additional samples on arrival of the adsorbent. This comparison may now be made and, subject to certain provisos, we believe the modification accurately reflects differences in riboflavin content within a series. With respect to absolute values, it is in accord with microbiological assay by means of *Lactobacillus casei*. It has, further, certain advantages: increased light stability, no adsorbent is needed and the riboflavin in the aqueous buffer exhibits approximately twice the fluorescence found in pyridine–acetic solution, with consequent decrease in the percentage reading error.

The Conner–Straub procedure is followed in detail in extraction and preparation of the sample, except that, in the case of fruits, 10 ml. of pectinol (1 g. in 25 ml.) is added per 50-ml. of sample, in addition to the clarase. The whole is then incubated at 45° for two hours. The pectinol is absolutely necessary for prunes, apricots, dates, etc., to produce a satisfactory solution. A 10–20 ml. aliquot is then heated to boiling with 5 ml. of 2% acetic, as in (1), made to volume, 50 ml., with buffer, and a 15-ml. aliquot treated for a minimum of three minutes with 1 ml. of potassium permanganate, and decolorized with 3 ml. of 3% hydrogen peroxide. The solution is then filtered and compared with buffered standards at *p*H 6.0 in a Coleman fluorophotometer. The B_2 filter for the exciting light (Hg arc) cuts out completely above 4900 Å. and for the fluorescent light, below 5100 Å. The cut-out is sharp, and, for the latter filter, the transmission rises from zero at 5100 to over 90% at 5400 Å. Quinine sulfate and thiochrome have no effect on the galvanometer with these filters, at least in the concentrations used. In the majority of plant extracts treated as above, there appear to be no other water-soluble fluorescent compounds in sufficient quantities to interfere, though trouble might be anticipated in those botanical families where anthraquinone glucosides occur. However, since we do not know the behavior of these compounds on Decalso or Supersorb, similar difficulties might arise with either method.

(1) With the Department of Home Economics.

(2) Conner and Straub, *Ind. Eng. Chem., Anal. Ed.*, **13**, 385 (1941).

(3) Supersorb, Florisil or Floridin is a Fuller's Earth; Decalso, a synthetic zeolite, obtainable through supply houses, Clarase (Takamine Laboratories, N. Y.) and Pectinol (Röhm and Haas, Philadelphia) are commercial enzyme preparations.