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BROM DERIVATIVES OF HYDROTHYMOQUINONE.*

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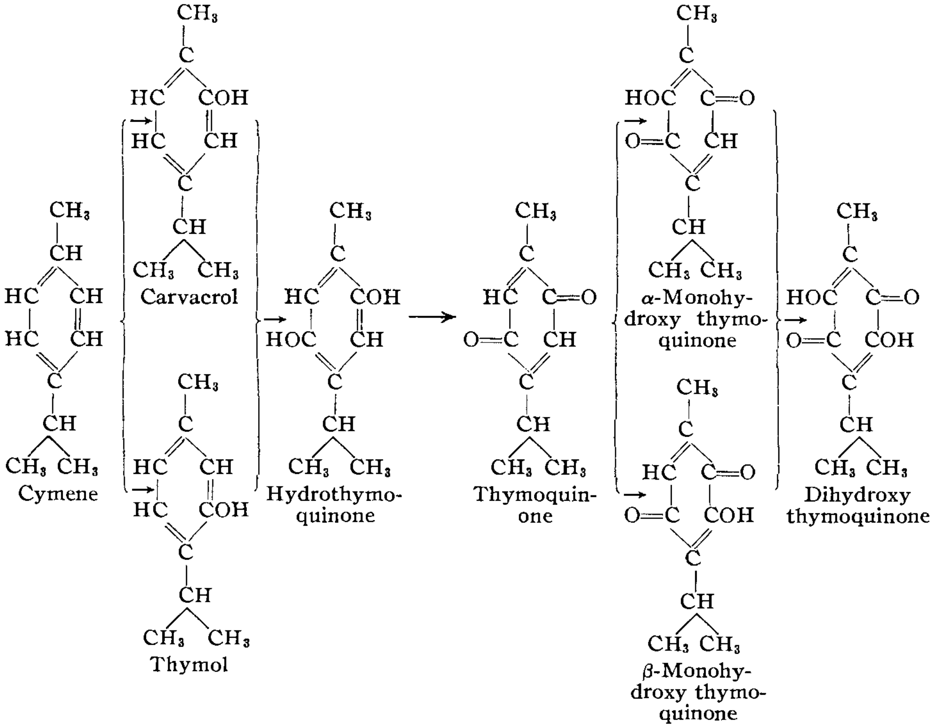
In the course of a bactericidal study of oxygenated derivatives of cymene, a striking group has been studied in connection with the phytochemical investigation of various species of the genus Monarda. The chemical relationship of its members can readily be understood by a glance at the following structural formulas:



It has been noted that to a certain extent an increase in the hydroxyl content resulted in a corresponding elevation of bactericidal potency (1). In other words,

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* Scientific Section, A. PH. A., Toronto meeting, 1932.

the phenol coefficients of hydrothymoquinone were found to be higher than those of either of the monatomic phenols. With this in mind, a more thorough study of the derivatives of hydrothymoquinone next suggested itself. Interest was directed more particularly to the halogen derivatives, especially in the light of the fact that the chloro and bromo compounds of both thymol and carvacrol showed unusual bactericidal efficiency.

The bromine containing compounds will be discussed first. On a previous occasion, the writer had made a preliminary study of the direct bromination of hydrothymoquinone and it was observed that oxidation was brought about with the resulting formation of bromine substitution products of thymoquinone. By the direct bromination of hydrothymoquinone with one, two, three and four moles of bromine, respectively, in acetic acid solution, the products which were obtained are compared below with the corresponding brom derivatives of thymoquinone:

1. The action of Br_2 resulted in the formation of 6-monobromthymoquinone (2) which crystallized in yellow needles that melted at 48° and that were soluble in alcohol, chloroform and benzene.
2. The action of 2 Br_2 resulted in the formation of 3,6-dibromthymoquinone (3) which crystallized in yellow needles that melted at 73° and were insoluble in cold alcohol, but soluble in hot alcohol, in cold ether, chloroform and acetone.
3. The action of 3 Br_2 resulted in the formation of the same tribromide (4) that had previously been obtained from thymoquinone, which crystallized in yellow laminæ that melted at 65°, and were insoluble in cold alcohol, but soluble in hot alcohol, also in ether and acetone.
4. Whereas the action of 4 Br_2 on thymoquinone had resulted in the formation of a monobromide (5) melting at 53° and of the 3,6-dibromide melting at 73°; the action on hydrothymoquinone gave the same yellow laminæ of the tribromide melting at 65° (see No. 3).

It becomes evident, therefore, that the preparation of the bromhydrothymoquinones will have to be brought about by some other method. A study of the literature reveals that the first mention of the mono- and dibromhydrothymoquinone was made by Schniter (2) and Schulz (6) in 1882, however, not as such but as the respective acetates. They prepared the 6-monobromhydrothymoquinone diacetate by treating thymoquinone with acetylbromide. In 1887, Schniter (7) effected the formation of 6-monobromhydrothymoquinone by the action of HBr on thymoquinone. However, he reported its melting point as 53°, whereas the melting point of 63–64° has been obtained in this investigation.

6-Monobromhydrothymoquinone.—*(a) Reduction with Sulphur Dioxide.*—The 6-monobromthymoquinone was prepared by the bromination of thymoquinone in chloroform solution at 5°, whereby yellow needles were obtained, which when recrystallized from 95 p. c. alcohol, melted at 48–49°. It has been observed that by treating 10-Gm. portions of thymoquinone with a mono-molecular equivalent (14.7 Gm.) of bromine, the mono compound only is formed. Larger quantities yield chiefly the dibromthymoquinone, m. p. 73° with low yields of the monobrom derivative.

5 Gm. of 6-monobrom thymoquinone were dissolved in a 75 p. c. alcohol. This solution was then saturated with SO_2 gas, the reaction mixture being kept at a temperature of from 40–50°. The solution was then allowed to evaporate

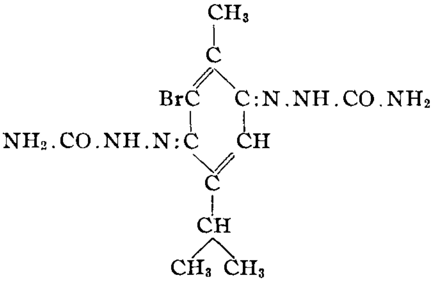
spontaneously, whereby the 6-monobromhydrothymoquinone formed as light orange plates in almost theoretical yield. These were thoroughly dried by pressing on a porous plate and were found to melt sharply at 63–64°. For further purification, this substance was gently boiled in alcoholic solution with one Gm. of Darco, a commercial activated charcoal. The filtrate yielded the same light orange plates, m. p. 63–64°. A bromine determination by the Carius method yielded 32.6 p. c., the theoretical bromine content being 32.6 p. c.

When acetylated with sodium acetate and acetic acid anhydride, white prisms, m. p. 91° (6), of the monobrom diacetate were formed.

(*b*) *Reduction with Zn and HCl.*—5 Gm. of 6-bromthymoquinone were suspended in 95 p. c. alcohol to which 20 cc. of concd. HCl had been added. To this was added zinc dust in portions of about 0.2 Gm. while the mixture was refluxed until all of the solid had been completely dissolved. The mixture was then refluxed for an additional half hour, the solution filtered through a hot water funnel, and the filtrate allowed to cool and evaporate spontaneously. A crop of white crystals was collected, which was recrystallized from hot water, and dried. 2.7 Gm. of hydrothymoquinone, m. p. 140–142°, were obtained. The zinc had evidently extracted considerable of the bromine. A dark brown solid separated out of the mother liquor, which probably was a mixture of 6-monobromthymoquinone, and a quinhydrone formed from unreacted 6-monobromthymoquinone and the hydrothymoquinone. This impure product melted between 85–110° and was readily soluble in alcohol, ether and acetone.

5 Gm. of dibromthymoquinone were then treated as described above, 2.9 Gm. of orange sheaths of the 6-monobromhydrothymoquinone, m. p. 62–64°, were first collected. The mother liquor yielded less than a gram of unreacted dibromthymoquinone, m. p. 73°. The crystal sheaths showed no change in melting point, when recrystallized from hot heptane. The diacetate melted at 91° indicating that a bromine atom had been abstracted.

Mention is also made here of the formation of 6-monobromhydrothymoquinone in an entirely different manner. The writer was studying the feasibility of separating a mixture of mono- and dibromthymoquinone by their respective monosemicarbazides. In doing so 5 Gm. of the above mixture were reacted with an approximate monomolecular equivalent of semicarbazide hydrochloride in 95 p. c. alcohol solution, the contents being refluxed for an hour. On cooling, 1.2 Gm. of a yellow crystalline material were collected by filtration, which melted sharply at 223° and which was identified as the 6-brom dicarbazide of thymoquinone, having the following configuration:



The mother liquor on further concentration yielded 3.6 Gm. of a light orange crystalline deposit, which when recrystallized from hot heptane melted at 62–64°. This substance was further established as 6-bromhydrothymoquinone by its diacetate, m. p. 91°.

Inasmuch as the reducing property of phenyl hydrazine can readily be explained by its liberation of hydrogen according to the equation

$$C_6H_5NH.NH_2 \longrightarrow C_6H_6 + N_2 + H_2 \quad (8)$$

the probability of semicarbazide acting as a reducing agent is not too far fetched. The reaction can possibly be explained in the following manner:

$$2\ H_2N.NHCONH_2 \longrightarrow 2\ NH_2CONH_2 + N_2 + H_2$$

3,6-Dibromhydrothymoquinone.—The literature does not describe this compound as such. Schulz (9), in 1882, prepared its diacetate, by brominating 6-brom diacetate of hydrothymoquinone in ether solution. He assigned to it a melting point of 121–122°, in contrast to 96–97° reported later on in this investigation.

(*a*) *Reduction with Zinc and HCl.* As has been previously described, when dibromthymoquinone was reduced with zinc and HCl, one bromine atom was abstracted to yield the 6-monobromhydrothymoquinone. In the light of these results, tribromthymoquinone was similarily treated to result in the formation of orange rhombic crystals, m. p. 60°. To make use of a mixture of dibrom and tribromthymoquinone, made available in the course of brominations of thymoquinone, the following products were identified from its reduction with zinc and HCl:

Orange rhombic plates, m. p. 59–60°. (Dibromhydrothymoquinone.)
Yellowish orange plates, m. p. 62–64°. (6-Bromhydrothymoquinone.)
Reddish prisms, m. p. 48–50°. (6-Bromthymoquinone.)

Dibromhydrothymoquinone is very readily soluble in alcohol, ether, acetone and heptane, whereas dibromthymoquinone is difficultly soluble in cold alcohol but readily so in hot alcohol, cold ether, acetone and heptane.

Bromine determinations (Carius) revealed as follows:

Found 48.7 p. c., calculated 49.3 p. c.

The diacetate, $C_{10}H_{10}Br_2(OCOCH_3)_2$, was obtained as white prisms when recrystallized from hot absolute alcohol, m. p. 96–97°. Schulz reported a melting point of 121–122° for this same substance, preparing it, however, by brominating the 6-bromdiacetate of hydrothymoquinone. When analyzed for bromine, the compound, m. p. 96–97°, contained 38.5 p. c.; calculated for $C_{10}H_{10}Br_2(OCOCH_3)_2$, 39.2 p. c.

(*b*) *Reduction with Sulphur Dioxide.* The dibromthymoquinone was then reduced by passing SO_2 into a hot alcoholic solution of the former. On cooling a fraction of a Gm. of the above material, m. p. 73°, was separated by filtration. The filtrate was allowed to concentrate spontaneously, whereupon handsome orange plates were obtained in almost quantitative yield. These were further purified by recrystallization from heptane, melting sharply at 60°.

(*c*) *Reduction with Hydrogen Sulphide.* By treating dibromthymoquinone with an ammoniacal solution of H_2S, a small crop of yellow crystals, m. p. 118–119° was formed, which as yet have not been investigated any further.

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WISCONSIN PHARMACEUTICAL EXPERIMENT STATION.

THE UNSAPONIFIABLE FRACTION FROM SPINACH FAT. II.

BY FREDERICK W. HEYL AND DONALD LARSEN.

In a previous publication (1) from this laboratory, the isolation of a hydrocarbon melting at 67.5–68.5° and two alcohols melting at 76–77° and 87–88°, respectively, was described. The second of these alcohols, of which we obtained but 0.2 Gm., appeared to be a glycol.

In recent papers (2) (3) it has been shown that the hydrocarbon is $C_{31}H_{64}$, hentriacontane.

The point was brought up by the English workers concerning the identity of the alcohol melting at 76–77° with ceryl alcohol (C, 81.67; H, 14.13) with which conclusion our previously reported results are at variance (C, 80.9; H, 14.2). The spinach which we extracted presents a highly complex mixture. It contained not only a preponderant amount of hentriacontane but also a glycol and the last substance appears to be absent from English spinach. The glycol for which we previously reported a m. p. of 87–88° has now been obtained purer and melts at 103.5°.

Elaborate effort has been made to definitely describe the monohydric alcohol and to identify it with certainty. This problem is greatly facilitated by the recent work of Pollard, Chibnall and Piper (4) who isolated pure *n*-hexacosanol from cocksfoot grass (Dactylis Glomerata). They reported that the alcohol melts at 79.7° and its acetate at 59.5–59.6°. Collison and Smedley-Maclean found for their ceryl alcohol from spinach 77–78° and for the acetate 61.5°.

Our further work shows conclusively that the spinach alcohols are a mixture of *n*-tetracosanol and *n*-hexacosanol with the former predominating. It is also shown that fractionation of the paraffin alcohols or of their acetates does not give the satisfactory analysis of this fraction which results finally from working with the corresponding acid mixtures.

Incidentally a new dextro-rotatory sterol has been isolated from the mother liquors of the less soluble spinasterol.