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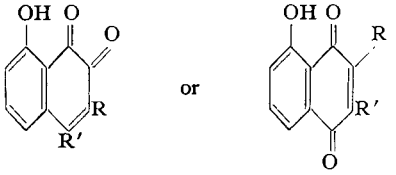
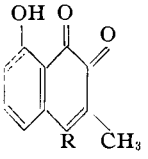
The Constitution of Celastrol, Part IV

*By Ole Gisvold**

In previous publications (1) it was shown that celastrol, a pigment found in the outer bark of the root of *Celastrus scandens*, has the formula $C_{22}H_{30}O_3$. It was postulated to be either a mono- or dialkyl substituted β- or α-naphthoquinone with one of the following tentative formulas, in which the sum of R and R′ equals $C_{12}H_{26}$.



Subsequent investigations on the constitution of this pigment indicate that it is a 3,4 - dialkyl - 8 - hydroxy - β - naphthoquinone. The alkyl group in position 3 appears to be a methyl group and the one in position 4, a homohydrogeranyl group ($-CH_2CH_2CH_2CH(CH_3)CH_2CH_2CH_2CH(CH_3)_2$), or possibly a hydrogeranyl group ($-CH_2CH_2CH(CH_3)CH_2CH_2CH_2CH(CH_3)_2$), as shown in the following formula:



The evidence presented in this paper together with that of Fieser and Jones (2) clearly indicates that the pigment is definitely a substituted β-naphthoquinone. In a previous paper by the author (1), the solubility of celastrol in bisulfite was not carried out to the best advantage, as shown by Fieser. Also the very small amount of a derivative prepared by a reaction of methyl celastrol with *o*-phenylenediamine left room for doubt that it was an orthoquinone. The author has since tried to prepare a derivative with *o*-phenylenediamine and also with substituted hydrazines with negative results.

Previous oxidative studies yielded little if any satisfactory quantities of identifiable fragments. The most successful of this type of investigation was that carried out with cold permanganate.

Samples of celastrol were ozonized in glacial acetic acid and the ozonide decomposed in the presence of hydrogen peroxide. This type of oxidative study yielded about 60% of an identifiable fragment; however, difficulty was encountered in attempting to purify the degradation product. The main degradation product responded to tests for a keto acid, but it failed to give a color reaction with ferric chloride. A 2,4-dinitrophenylhydrazone was readily obtained in a pure form suitable for analysis, the results of which corresponded to the formula $C_{25}H_{32}O_7N_4$, m. p. 192° C.

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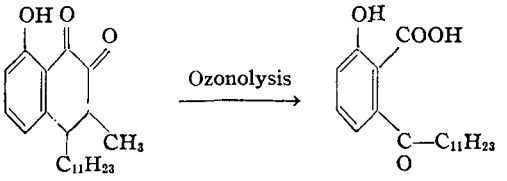
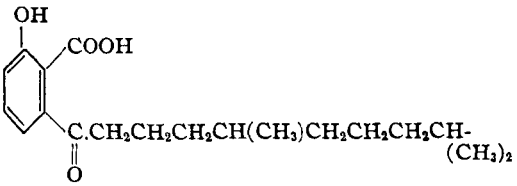
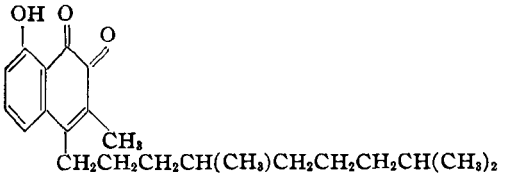
Celastrol therefore lost three carbon atoms upon ozonolysis and one could postulate the formation of a keto acid, as follows:



The keto acid obtained upon ozonolysis had a specific rotation in alcohol of +22.1. The optical activity therefore resides in the side chain $-C_{11}H_{23}$ and one could postulate that it is a homohydrogeranyl group, although the possibility of a hydrogeranyl group cannot be excluded. The keto acid and celastrol would then possess the following formulas:

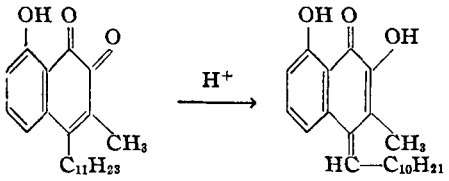


Keto Acid



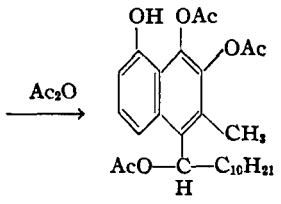
Celastrol

In a previous paper (1) it was reported that celastrol and methyl celastrol yielded yellow diacetates. These acetates were prepared by heating the substance in question with acetic anhydride and a relatively large quantity of pyridine. A continuation of acetylation studies has revealed some interesting observations. Sodium acetate and acetic anhydride yielded a colorless diacetate which turned yellow at the melting point, 241° C., which was the same as that of the previously prepared yellow diacetate. This colorless acetate turned yellow upon standing. In test acetylations, small amounts of pyridine gave a colorless acetylation mixture, whereas large amounts of pyridine gave a yellow acetylation mixture. Acetylation in the cold with acetic anhydride containing a trace of concentrated sulfuric acid (Thiele reagent) gave a permanently colorless abnormal triacetate which melted at 100–101° C. According to Fieser (3), quinones other than those having a conjugated system with hydrogen on the terminal carbon atom $(-CH=C-C=O)_1$, this behavior has been noted only with 4-alkyl-1,2-naphthoquinones, such as the 4-methyl and 4-benzyl derivatives. With celastrol the reaction could proceed through a tautomerism to the aci-form and a Thiele addition.



Celastrol

Celastrol (Aci-form)

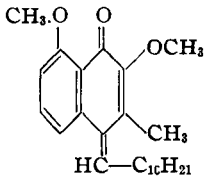


Celastrol Triacetate

The abnormal triacetate of methyl celastrol has also been prepared and found to be colorless.

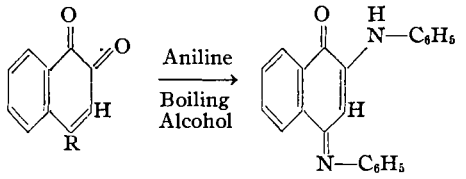
Fieser (4) has prepared the stable aci-form of certain β-naphthoquinones. Employing his technique an attempt was made to prepare the aci-form of celastrol. Celastrol was dissolved in cold concentrated sulfuric acid and after some time the deep red solution when poured into ice water yielded a yellow precipitate. A yellow solution was obtained when the precipitate was dissolved in ether, which indicated the presence of the aci-form. However, when attempts were made to crystallize this form the solution gradually assumed the characteristic red color of celastrol and no aci-form was isolated.

The aci-form of celastrol no doubt exists in the yellow dimethyl celastrol, a compound previously prepared (1) and reported. Its formula is shown as follows:



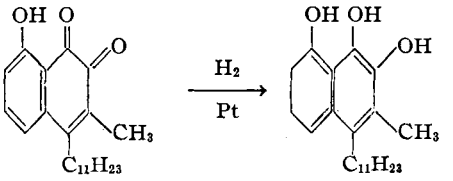
Dimethyl Celastrol

Fieser and Bradsher (5) have shown that the action of an excess of aniline in boiling alcohol on the 4-methyl, 4-benzyl, and 4-dicarbethoxy derivatives of 1,2-naphthoquinones resulted in each case with the displacement of the 4-substituent and formation of 2-anilino-1,4-naphthoquinone-4-anil. This substance was identical with the reaction product resulting from the interaction of β-naphthoquinone with aniline.



This reaction was carried out with celastrol with hopes that the alkyl group in position 4 in celastrol would be eliminated. This could aid in the characterization of this part of the molecule. However, celastrol was the only substance that could be recovered from the reaction mixture even after prolonged heating.

Reduction of celastrol in alcohol with platinum black as a catalyst yielded the corresponding phenol as dihydrocelastrol. Dihydrocelastrol crystallized as colorless needles from alcohol and melted at 177° C. In solution it was readily oxidized by air and even in the solid state it was slowly oxidized to the original quinone.



Celastrol Dihydrocelastrol

EXPERIMENTAL

Ozonolysis of Celastrol.—Five grams of celastrol were dissolved in 100 cc. of glacial acetic acid and the theoretical quantity of ozone passed through this solution at a low temperature.[1] The ozonide was decomposed by boiling in the presence of hydrogen peroxide. The solvent was removed under reduced pressure. The residue was taken up in ether, washed with water and the resulting solution evaporated to dryness. The residue thus obtained was extracted with hot petroleum ether. No detectable amount of material was soluble in petroleum ether. The residue weighed about 3 Gm. and was best purified by fractional crystallization from anhydrous ether and petroleum ether. The purest fraction was very pale yellow in color, melted at 166–167° C. and had a specific rotation of +22.1° in alcohol. It was soluble in sodium bicarbonate, formed a hydrazone, but gave no color test with ferric chloride. The substance is therefore a keto acid.

2,4-Dinitrophenylhydrazone of the Keto Acid.—This derivative was prepared by dissolving the above keto acid in alcohol, and sufficient solution of 2,4-dinitrophenylhydrazine (1.5% in 10% sulfuric acid containing 25% of alcohol) was added to equal about twice the theoretical quantity needed. Any turbidity encountered at this point was eliminated by the addition of alcohol. The mixture was heated on the steam bath for about 10 min. A small quantity of ether was added and the reaction mixture allowed to stand overnight. A crystalline brick-red hydrazone separated out. The derivative was collected on a fritted glass funnel, washed free from acid with water and then washed once each with alcohol and ether, m. p. 192° C. The derivative was soluble in alkali carbonate, giving a deep red solution.

Analysis ($C_{25}H_{32}O_7N_4$): Calcd.: C, 60.00; H, 6.40. Found: C, 59.81, 60.07; H, 6.36, 6.35.

ACETYLATION EXPERIMENTS

Thiele Reaction.—One gram of celastrol was dissolved in about 5 cc. of acetic acid to which was added one drop of concentrated sulfuric acid. The reaction mixture was allowed to stand overnight at room temperature during which time the solution became colorless. An excess of sodium acetate was added and the solvent removed under a vacuum. The residue was extracted with anhydrous ether and the colorless derivative fractionally crystallized from a mixture of anhydrous ether and petroleum ether. The purest fraction melted at 100–101° C. and analysis for acetyl value showed it to be an abnormal triacetate.

Analysis ($C_{28}H_{34}O_7$): Calcd.: CH_3CO, 26.4% (for 3 acetyl groups). Found: 28.0%.

The Thiele acetylation when applied to methyl celastrol also gave a colorless abnormal triacetate.

Analysis ($C_{29}H_{40}O_7$): Calcd.: CH_3CO, 25.7% (for 3 acetyl groups). Found: 24.8%.

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[1] By Dr. Hill, through the courtesy of Dr. Henne at Ohio State University.

The derivative is exceedingly easily hydrolyzed by dilute aqueous alkali in the cold and the solution quickly assumes an intense deep red color.

Acetylation with Sodium Acetate.—One-half gram of celastrol was acetylated by warming it on the steam bath for 1 hr. with an excess of acetic anhydride in the presence of sodium acetate. The mixture was allowed to stand overnight, after which time it became colorless. If the mixture was heated again it became yellow and upon cooling and standing it became colorless again. The excess reagent was removed by an air current in the hood. The residue was extracted with anhydrous ether, mixed with petroleum ether and the almost colorless derivative fractionally crystallized from this mixture. This acetate melted at 241° C., the same m. p. as that of the yellow acetate previously prepared and reported. This acetate although almost colorless turned deep yellow just before melting. Upon standing in a vial this acetate gradually turned a deep yellow.

Acetylation with Pyridine.—In test experiments, a small amount of pyridine in acetic anhydride gave a colorless solution with celastrol. However, when a large amount of pyridine was used, the reaction mixture assumed a deep yellow color.

Reaction with Aniline.—One gram of celastrol was dissolved in 15 cc. of warm absolute alcohol, 2 cc. of aniline was added and the solution was warmed on the steam bath for 1 hr. Upon standing and cooling, 0.5 Gm. of celastrol crystallized out. Further recovery of the reactants resulted only in the recovery of celastrol itself. These celastrol crystals were dark red in color. Prolonged heating of the reaction mixture gave the same results. Under the conditions of the above experiment, no reaction of celastrol with aniline takes place.

Reduction of Celastrol.—One-half gram of celastrol was reduced with platinum black in alcohol at 25° C. and 40 lbs. pressure. The reaction flask was removed, stoppered and allowed to stand for several weeks, after which time the reduction product separated as colorless clusters of fine needles which were quite insoluble in methyl and ethyl alcohols, ether, chloroform and petroleum ether. They were very soluble in pyridine; however, when in solution rapid oxidation took place and the solution soon became colored. Difficulty was experienced in separating the crystals from the platinum catalyst. Reduced celastrol melted at 177° C. Even in the solid state this reduction product, which could be called dihydrocelastrol (the reduced form of the quinone), was slowly oxidized by air to the orange quinone form.

Attempted Preparation of Aci-form of Celastrol.—One-half gram of celastrol was dissolved in 5 cc. of sulfuric acid at 0° C. and after 3 min. the deep red solution was poured into water. The yellow precipitate was dissolved in ether, washed with water and attempts made to crystallize the resulting product failed. That the aci-form was present was evidenced by the yellow solution, whereas an equal amount of celastrol would have made a red solution. Failure to crystallize the aci-form was due to the transformation of the aci-form to the quinone form during the process of attempted crystallization.

SUMMARY

1. Celastrol upon ozonolysis yields a keto acid which, according to the analysis of the 2,4-dinitrophenylhydrazone, has the formula $C_{19}H_{28}O_4$.

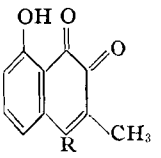
2. Celastrol forms a colorless abnormal triacetate characteristic of certain β-naphthoquinones when acetylated with the Thiele reagent.

3. Celastrol when acetylated with sodium acetate and acetic anhydride forms a colorless diacetate which turns deep yellow at the melting point or upon standing.

4. No reaction takes place with boiling alcohol and aniline.

5. Dihydrocelastrol, which is colorless, has been prepared in a crystalline form.

6. The evidence presented in this paper together with that of Fieser and Jones, (2) clearly indicates that celastrol is a 3,4-dialkyl-8-hydroxy-β-naphthoquinone with the following tentative formula, in which R may possibly be $-CH_2CH_2CH_2CH(CH_3)CH_2-CH_2CH_2CH(CH_3)_2$ or $-CH_2CH_2CH(CH_3)-CH_2CH_2CH_2CH(CH_3)_2$:



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