ever, when the dog was removed from the cage, it was observed that he staggered slightly as he walked. At this time the same dose of the extract previously given was administered by means of a stomach tube. The first symptoms of poisoning noted were intense salivation and vomiting. These appeared shortly after the administration of the poison. The heartbeat appeared to become somewhat slower and irregular, but it was difficult to make an accurate count. The dog attempted to vomit on several occasions but was unable to do so. At 27 minutes after administration, the animal experienced severe convulsions and died within several minutes. Postmortem examination revealed the heart stopped in systole. It is believed that heart action and respiration ceased almost simultaneously.

SUMMARY

1. The structure of the inner scale of the bulb of red squill was studied microscopically.

2. Powdered red squill and powdered white squill were compared under the microscope. The presence of fragments of red pigmented tissue in the red squill powder served as the only diagnostic difference between the two powders.

3. The active rat-killing principle was extracted using 80% ethyl alcohol as the solvent. This, on subsequent purification with animal charcoal, ether and petroleum ether, yielded a product which caused death in a male rat when fed at the rate of 4 mg./Kg.

4. The physiological action of the rat-killing principle on rats and dogs was observed.

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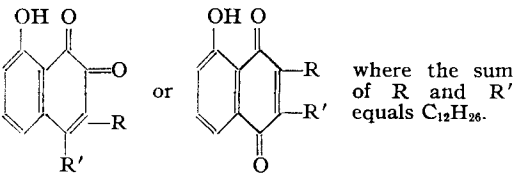
Wilhelm Ostwald (1853–1932) was awarded the Nobel Prize for Chemistry in 1909 in recognition of his work on catalysis and for his investigations in the fields of chemical equilibria and reaction velocities.

The Constitution of Celastrol—Part III

*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 $C_{22}H_{30}O_3$. One oxygen was reported present as a hydroxyl group and the remaining two oxygens as possibly being in the form of an ortho quinone. Subsequent investigations on the constitution of this pigment indicate that it might possibly be an alkyl substituted 5-hydroxy-1, 4-naphthoquinone with the following tentative formulas



where the sum of R and R' equals $C_{12}H_{26}$.

Permanganate oxidation of celastrol yielded a small quantity of a crystalline material that was best characterized as 3-hydroxy phthalic acid. Attempts to obtain more of this compound or its methoxy derivative by oxidation with chromic acid did not prove successful. However, all crude oxidation materials gave a positive fluorescein test, with resorcinol when tested for phthalates.

Celastrol does not form a water-soluble bisulfite addition product and only a minute quantity of a phenazine. It can be reduced with sulfurous acid and reoxidized by atmospheric oxygen after the removal of sulfur dioxide.

Celastrol is orange and, according to Hooker, (2) 2 hydroxy naphthoquinones which have a side chain in position, 3 are yellow if a double bond is present in the β,γ-position and red to orange if the double bond is in the α,β-position. Ultimate analysis of celastrol indicates that it does not have a double bond in the side chain. This is also strengthened by the fact that celastrol when reduced with Raney nickel at 190° in alcohol is converted to its original color upon

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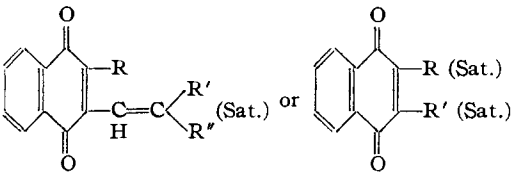
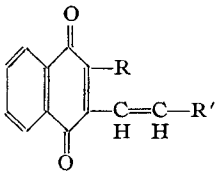
spontaneous oxidation in air. If a double bond were present in the α,β-position which was contributing to the orange-red color of celastrol, it would have to be inert to the drastic hydrogenation to which it has been subjected.

Celastrol or methyl celastrol fail to give a violet color test with 1 per cent sodium methoxide. Compounds with the following constitution give no color or at best a weak blue (3).



α-Naphthoquinones with the following constitution give an intense purple or blue color test with 1 per cent sodium methoxide (4).



According to Fieser (5), those naphthoquinones examined which have no activating double bond or phenyl group attached to the α-carbon atom of the side chain do not undergo an abnormal acylation. Although celastrol apparently conforms to the above requirements, nevertheless it forms a yellow acetate which is indicative of the abnormal acylations that is characteristic of the naphthoquinones investigated for this property.

When tested for its antihemorrhagic activity, celastrol proved to be entirely negative in effect in doses as high as 100 mg. per Kg. Methyl celastrol was inert in all doses below 25 mg. per Kg., although a slight effect was evident at the 25-mg. dose.[1]

EXPERIMENTAL

Oxidation with Potassium Permanganate.—Five grams of celastrol were oxidized with aqueous alkaline permanganate according to R. Kuhn and A. Deutsch (6). All attempts to obtain a crystalline product by crystallization methods failed. The following technique overcame this difficulty. Purified sand was impregnated with the oxidized material and then extracted with Skelly-solve *B*. The Skelly-solve *B* soluble fraction was crystallized from dry ether. The crystalline material obtained in this way had an aromatic odor which was characteristic of the material still remaining upon the sand. The material when crystallized from water was odorless, colorless and, when dried, melted at 245° C. It was difficult to obtain a sharp melting point. This material could be readily sublimed and the crystalline sublimate melted at 242–244° C. It gave a positive fluorescein reaction.

Analysis:

$C_8H_6O_5$	Calc.	C	52.6	H	3.3
	Found	C	52.20	H	3.14
			52.22		2.90

Molecular weight, rast 190

No color was obtained when it was tested with ferric chloride. 3-Hydroxyphthalic acid is reported as melting at 161° to 163° C. and its anhydride at 198° to 199° C. (7). It is reported as melting at 244° C. in the "Handbook of Chemistry and Physics."

Oxidation of Celastrol with Chromic Acid in Glacial Acetic Acid.—In an attempt to prepare more phthalic acid or a phthalic acid derivative, celastrol was oxidized with chromic acid in boiling acetic acid (8). One gram of celastrol was refluxed with 3 Gm. of chromic acid in glacial acetic acid for about one and one-half hours. No crystalline material could be obtained from the sodium bicarbonate soluble fraction of the oxidized material. However, it gave a positive fluorescein reaction. Successive reoxidations of the oxidized material by increasing the amounts of chromic acid did not lead to the successful isolation of a crystalline substance. In fact, such treatment resulted in the complete loss of any ether-soluble material.

Oxidation of Methyl Celastrol with Chromic Acid in Glacial Acetic Acid.—The same procedure described above was used in the oxidation of methyl celastrol. No crystalline oxidation substance could be obtained from the oxidation material. It gave a positive fluorescein reaction. Successive reoxidations of the oxidized material by increasing the amounts of chromic acid did not lead to the successful isolation of a crystalline substance. In fact, such treatment resulted in the complete loss of any ether-soluble material, but this destruction proceeded at a slower rate than with celastrol.

Reductive Acetylation.—Methyl celastrol was mixed with zinc dust, acetic acid and acetic anhydride. The mixture was warmed on the steam-bath for two hours. The reaction mixture remained colorless as long as the evolution of hydrogen took place. The reaction mixture was filtered and the slightly yellow filtrate intensified in color upon exposure to air and removal of the solvent.

[1] Marvin R. Thompson personal communication through the courtesy of the Warner Institute for Therapeutic Research.

This derivative separated from anhydrous ether in the form of a crystalline powder which melted at 210° C.

Analysis: CH_3CO

$C_{27}H_{38}O_5$ Calc. for 2 acetyl groups 19.50 per cent
Found 19.68, 22.67, av. 21.17 per cent

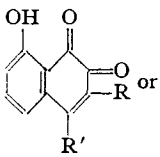
Reaction with Bisulfite.—Celastrol was found to be insoluble in a saturated solution of sodium bisulfite and it cannot be extracted from an ether solution with this reagent.

Reaction with Sulfurous Acid.—An alcohol solution of either methyl celastrol or celastrol became colorless upon the addition of sulfurous acid. The solutions became colored again upon removal of the sulfur dioxide and aeration of the mixture.

Reaction with Orthophenylenediamine.—Methyl celastrol was refluxed with *o*-phenylenediamine and hydrochloric acid in a hydro-alcoholic solution. The reaction mixture was diluted with water and extracted with ether. The ether solution was extracted with dilute alkali to remove any unreacted material. A very small amount of yellow needles was obtained from the ether solution. These needles melted with decomposition at 275° to 285° C.

SUMMARY

The evidence presented in Papers I and II together with subsequent investigations upon the constitution of celastrol indicates that it has the formula $C_{20}H_{32}O_8$. It is either a mono- or dialkyl substituted β- or α-naphthoquinone of one of the following tentative formulas:



where the sum of R and R′ equals $C_{12}H_{26}$.

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A postage stamp in honor of the famous botanist, Karl von Linné has been issued by Sweden.

Assay of a Variety of Vitamin B_1 Preparations by the Fluorophotometric Method

*By J. W. Cole, W. S. Jones and W. G. Christiansen**

Until recent years one has had to rely on biological methods for the estimation or determination of vitamin B_1. The disadvantages of such a situation are obvious; the cost of biological assays and the time required became factors of considerable magnitude in investigational work. The isolation, identification and synthesis of thiamin have made possible the development of rapid physiochemical methods for the estimation of vitamin B_1. While the status of these chemical methods is at present not such that they can replace the biological assay as a basic standard for establishing the B_1 potency of a product and while it is questionable as to whether that ever should be so, because the products being dealt with are intended to produce certain biological responses or effects when fed to humans (or animals), the very great usefulness of a rapid, reliable chemical method for investigational work, process control, preliminary testing so as to reduce to a minimum the amount of biological testing, stability under storage conditions, etc., is readily appreciated.

Thiamin, in both the free and phosphorylated form, is oxidized, respectively, to the free and phosphorylated thiochrome by potassium ferricyanide in alkaline solution. The free thiochrome is readily extracted by isobutanol and gives a violet-blue fluorescence in ultraviolet light. The phosphorylated thiochrome is not extracted by isobutanol. However, when the thiamin in the material to be tested exists wholly or partially in the phosphorylated form, one can by enzymatic hydrolysis, as the first step in the assay, convert the phosphorylated form into free thiamin and thus make possible the recovery of all the thiamin in the form of free thiochrome for estimation

* Control and Development Divisions, Chemical and Pharmaceutical Laboratories. E. R. Squibb & Sons, Brooklyn, N. Y.