Celastrol, Spectrographic Characterization and Color Tests*

By L. F. Fieser and R. N. Jones

The ultraviolet absorption spectra (Fig. 1) were determined in ethanol solution and the log E molar values were calculated on the assumption of the formula $(C_{22}H_{30}O_3)_4 \cdot C_6H_{14}O$ for the celastrol sample and $C_{23}H_{32}O_3$ for methylcelastrol. Celastrol shows an intense, broad absorption band with a maximum at 422 mμ (log E = 3.84) and a shoulder at 256 mμ (log E = 3.8). The spectrum of methylcelastrol is similar, having a broad rounded band at 426 mμ (log E = 3.95) and a shoulder at 260 mμ (log E = 3.8).

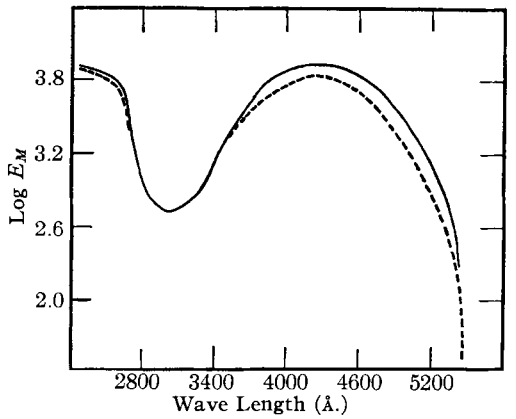

Fig. 1.—Absorption Spectra of Celastrol and Methylcelastrol in Ethanol.
- - - - - - - Celastrol
——————— Methylcelastrol

The absorption curves shown in Fig. 1 are wholly unlike those characteristic of α-naphthoquinone and its alkyl derivatives, but show one significant point of correspondence with the curves for substituted β-naphthoquinones. Cooke, Macbeth and Winzor (1) observed that a number of β-naphthoquinones show a rounded absorption band centering at 403–443 mμ, depending on the number and nature of the substituents, with log E values in the range of from 3.27 to 3.30 (see also Morton and Earlam (2)). Cooke, Macbeth and Winzor considered this distinctive band a characteristic mark of the β-naphthoquinonoid structure, but they did not emphasize the fact that many hydroxylated α-naphthoquinones exhibit a very similar band in the same region (3). Thus juglone (one OH) has a broad band at 425 mμ (log E = 3.7), isonaphthazarin (two OH groups) shows one at 445 mμ (log E = 3.3), and hydroxydroserone (three OH groups) has a maximum at 488 mμ (3.83). This broad band, which is completely absent from the spectrum of α-naphthoquinone (3), thus appears on the introduction of one hydroxyl group and is shifted progressively to longer wave lengths on increasing the number of such substituents. The distinctive band, however, is shown only by the free hydroxy compounds and disappears on replacement of the labile hydroxylic hydrogen atom. Thus acetylation of the hydroxyquinones results in a complete reversion to the α-naphthoquinonoid spectrum. The band shown by celastrol at 422 mμ thus might be indica-

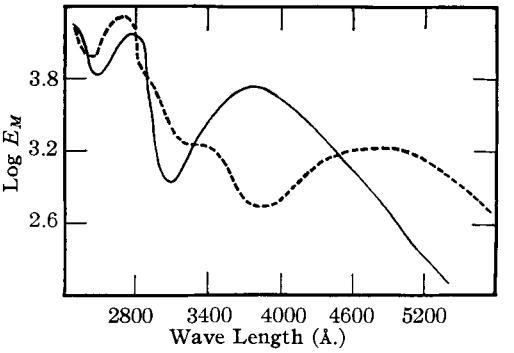

Fig. 2.—Absorption Spectra in Ethanol of 7-Hydroxy-1,2-naphthoquinone (Maxima in mμ and log E_{molar} Values: 270 (4.32); 300 (3.68 (Inflection)); 340 (3.23); 485 (3.22)) and 6-Hydroxy-1,2-naphthoquinone (278 (4.16); 366 (3.74)).
- - - - - - - 7-Hydroxy-1,2-naphthoquinone
——————— 6-Hydroxy-1,2-naphthoquinone

tive of either a β-naphthoquinonoid or a hydroxy-α-naphthoquinonoid structure, but the curve for the methyl ether provides a means of distinguishing between the two possibilities. The fact that with methylcelastrol the rounded band does not disappear but persists in essentially the original form affords a strong indication of the β-naphthoquinonoid character of the natural pigment. That the hydroxy compound and its methyl ether are so similar finds some analogy in the observation (1) that lapachol methyl ether has a spectrum in which the long wave length band is not so pronounced as in lapachol but is not affected to the same extent as in the acetate.

In the region of shorter wave length, the spectrum of celastrol departs considerably from that associated with such known β-naphthoquinones as have been studied (1, 3, 4), the chief point of difference being in the absence of a narrow band of intensity far superior to that of the broad long wave length band.

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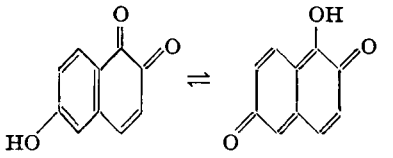
Thus β-lapachone shows such a band at 256.5 mμ (log E = 4.45), together with shoulders at 282 mμ (3.98) and 333 mμ (3.24) (1), while celastrol shows only a low intensity shoulder at 256 mμ. The difference may possibly be associated with the presence of the hydroxyl group, and for comparison we examined the 6- and 7-hydroxy derivatives of 1,2-naphthoquinone (Fig. 2). Spectrographically, 7-hydroxy-1,2-naphthoquinone closely resembles β-lapachone, while the 6-isomer is so widely different as to suggest the possibility of a tautomerism:

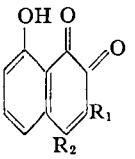


This is not without some analogy (5, 6). Clearly both compounds differ sufficiently from celastrol to warrant the conclusion that the pigment cannot be a 1,2-naphthoquinone having a hydroxyl group at either the 6- or 7-position. Unfortunately no other isomers are available for study as possible model compounds.

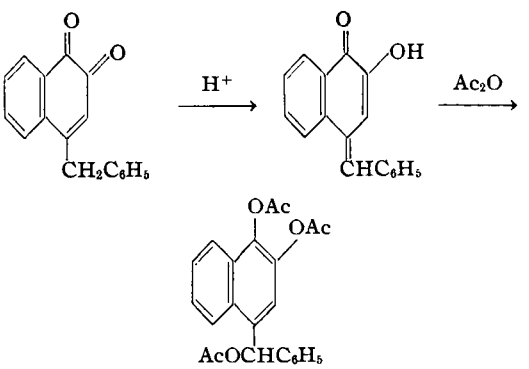
A β-naphthoquinonoid structure would be consistent with the colors of celastrol (red) and of methylcelastrol (orange), and also with the following observations concerning the behavior of the quinones toward sodium bisulfite. Although Gisvold (7) observed no reaction between celastrol and aqueous bisulfite, a prompt reaction occurs in an aqueous alcoholic medium. When an orange-red solution of celastrol in alcohol is treated with a saturated aqueous solution of sodium bisulfite, the pigment is at first precipitated but then rapidly dissolves to give a completely colorless solution. When a suspension of methylcelastrol is treated similarly with aqueous bisulfite solution the orange material soon dissolves to a colorless solution. On neutralization of the bisulfite with sodium carbonate the orange pigment is slowly reprecipitated. This characteristic behavior provides chemical evidence of an *ortho*-quinonoid structure.

Quinones having a hydroxyl group ortho to one of the quinone carbonyl groups give an intense coloration when treated with Dimroth's boroacetic anhydride reagent (5, 8), while other hydroxyquinones as a rule give no more color than when treated with acetic anhydride alone. Thus the Dimroth test is negative with 6- and 7-hydroxy-1,2-napthoquinone, while juglone (5-hydroxy-1,4-naphthoquinone) gives a positive orange-red test. Celastrol gives a light orange-yellow color with acetic anhydride alone, but on adding the Dimroth reagent a deep red color develops in the cold. This indicates that the hydroxyl group most probably is at the 8-position, although the 3-position may also be a possibility. It may be noted that the red color and high melting point (205° C.) of celastrol find some analogy in the properties of 6- and 7-hydroxy-1,2-naphthoquinone, which are orange-red and red, respectively, and melt at 165° C. and at 204° C. (9).

Another significant color test is that of Craven (10). Naphthoquinones having a free position in the quinonoid ring when treated in alcoholic solution with ethyl cyanoacetate and ammonia give an intense blue coloration, and the test is negative if both quinonoid positions are occupied by substituent groups. It has now been found that the test is applicable to *ortho* as well as *para* quinones. 2,6-Dimethyl-1,2-naphthoquinone and 4-benzyl-1,2-naphthoquinone both give intense blue solutions, while with the fully substituted β-lapachone the test is negative. With celastrol there is no sign of a blue color in the Craven test and instead an unusual phenomenon is observed. The substance dissolves in alcoholic ammonia with a deep reddish color and on adding ethyl cyanoacetate the color promptly fades and a colorless solution results. That the presence of a hydroxyl group does not interfere is shown by the observation that plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone) gives an intense purple-indigo color in the Craven test. Furthermore, methylcelastrol gives a negative Craven test and undergoes no apparent change. It may be concluded that both the 3- and 4-positions are occupied by substituents, and the foregoing observations may be interpreted in terms of the following tentative formula for celastrol:



Confirmatory evidence is found in the observation that when celastrol is treated in the cold with acetic anhydride containing a trace of concentrated sulfuric acid (Thiele reagent) the initial yellow color slowly disappears and the solution becomes completely colorless. Among quinones other than those having a conjugated system with hydrogen on the terminal carbon atom (—CH=C—C=O), this behavior has been noted only with 4-alkyl-1,2-naphthoquinones, such as the 4-methyl and 4-benzyl derivatives (11, 12). With the benzyl compound the reaction has been shown to proceed through a tautomerism to the aci-form and a Thiele addition:



A similar reaction may be responsible for the bleaching of the color in the Craven test. It was noted also that on extracting celastrol from an orange ethereal solution with soda, acidifying the aqueous solution and extracting with ether, a pure yellow solution was obtained. This again suggests the tautomerism of a 4-alkyl-1,2-naphthoquinone.

From Gisvold's observations (13) it is evident that the alkyl groups R_1 and R_2 must both be fully saturated. An attractive speculation is that the group R_1 is methyl, for a methyl group occupying the same position is present in the natural naphthoquinone pigments plumbagin, phthiocol, hydroxydroserone and vitamins K_1 and K_2. In such a structure, furthermore, the methyl and hydroxyl groups would occupy the same relative positions as in plumbagin. The group R_2 which, in view of the optical activity of the pigment (14), appears to have a branched chain, may be a hydrogeranyl or a homohydrogeranyl group.

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U. S. Pharmacopoeial Convention

Statement of Income and Expense for the Second Year of the 1940–1950 Decennial Period from May 1, 1941, to April 30, 1942*

The accounts of the U. S. Pharmacopœial Convention, covering the year ending April 30, 1942, have been audited by R. G. Rankin and Company, Certified Public Accountants of New York and Washington. At the direction of the U. S. P. Board of Trustees, copies of the Statement of Income and Expense, and of the Statement of Cash Receipts and Disbursements as prepared by the auditors are attached, together with supplemental data giving further details with respect to disbursements, under the heading Revision and Research.

As would be expected, the sales of the U. S. P. and its Supplements during the past year have been lower than those of the previous year. With the expectancy of the appearance of a new pharmacopœia, sales would naturally be at low ebb. The decrease noted in the expense of printing and binding might naturally be expected to follow the decrease in sales over this period.

An increase in Administration expenses for the year is explained by extra clerical work, largely in connection with the revision of the Constitution and By-Laws, by a meeting of the Constitution and By-Laws Committee in Detroit, and by a meeting of Dean Rogers and Dr. Bunce of the Committee with the Board of Trustees in New York City, together with several meetings of the Board and the interest on bank loans and miscellaneous general expenses.

Convention expense increases are notably due to the publication and distribution of the Proceedings of the 1940 decennial meeting as noted under Printing and Binding, and the general expenses which include an honorarium of $500.00 to Secretary Warren and the expenses of the 1942 meeting at Cleveland including counsel fees for the year.

The additional detailed statement regarding Revision and Research expenses which is included with this report, readily explains the decrease in revision expenses and the slight increase in those classified as research, when compared to the similar statement covering the activity of the previous year of the decade.

For the U. S. P. Convention,
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For the U. S. P. Board of Trustees,
E. F. KELLY, *Chairman*
ADLEY B. NICHOLS, *Secretary*

*Copies of this statement may be obtained by writing to the Secretary, Adley B. Nichols, 43rd St. and Kingsessing Ave., Philadelphia, Pa.