

intervals following ingestion. The results obtained are shown in Table II.

#### SUMMARY

1. Rabbit *A* showed a lowest blood sugar value of 94.5 mg./100 ml. and a highest value of 131.6 mg./100 ml., using 2 different doses and two different extracts. These figures are within the range of normal blood sugar values for *A* as indicated in Table I.

2. Similarly the lowest and highest values obtained for rabbits *B*, *C* and *E* also fall within the normal values for each.

3. Rabbit *D*, although showing values greater than the normal, did not give any below the normal.

#### CONCLUSION

These observations seem to indicate that oral administrations of our aqueous extracts of Devil's Club Root have no hypoglycemic effect upon the blood of rabbits which have been starved for 15 to 24 hours.

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## The Constitution of Celastrol —Part II

By O. Givold\*

A preliminary report on the constitution of celastrol, a pigment obtained from *Celastrus scandens*, was given in a previous paper (1). Celastrol crystallizes from organic solvents in the form of ruby-red cubes. The following derivatives were reported: methyl celastrol (orange needles), acetyl celastrol (yellow), methyl acetyl celastrol (yellow) and acetyl methyl celastrol (yellow). Of the derivatives prepared, the methylated compound (prepared by methylation with diazomethane) was the only one to be obtained in a good crystalline form. Its methoxyl content was 9.01 (av.) which indicated the introduction of one methoxyl group. The molecular weight by the Rast method was 347. Celastrol and methyl celastrol gave a green color with ferric chloride whereas the acetylated derivatives did not. Celastrol appeared to be reversibly reduced at 190° C. with Raney's nickel. Ultimate analysis of celastrol indicated the formula  $C_{23}H_{36}O_3$ . It will be shown in this paper that this formula is not correct because not all the solvent of crystallization could be removed by drying at 100° C. over phosphorus pentoxide in a vacuum. Ultimate analysis of the acetate indicated the formula  $C_{26}H_{34}O_5$ .

Subsequent investigations on the constitution of celastrol indicate that no carboxyl groups are present in the molecule. In addition to the introduction of one methoxyl group, it will be shown that two acetyl groups can be introduced, thus accounting for the presence of three oxygens. One or both of the acetyl groups must be hydrolyzed very rapidly because alcoholic alkali causes an immediate development of a deep red color. It appears that a second methoxyl group can be introduced into the molecule by means of dimethyl sulfate and alkali. This dimethoxyl compound is yellow in color. Catalytic reduction of celastrol and methyl celastrol with platinum black at 40-lbs.

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pressure and ordinary temperatures gives colorless or nearly colorless products that give a green color with ferric chloride and are oxidized by atmospheric oxygen to red compounds again. Celastrol is unstable in the presence of potassium hydroxide and air. It is oxidized instantly to a pale yellow compound by means of hydrogen peroxide and alkali and is reversibly reduced by means of sulfurous acid.

The results of investigations to date indicate that celastrol has the formula  $C_{22}H_{30}O_3$ . One hydroxyl group is acidic enough to be methylated by means of diazomethane. The remaining two oxygen atoms appear to be in the form of an ortho quinone. One or both carbonyl groups evidently can be forced into an enolic form by acetylation as evidenced by the mitigation of color.

#### EXPERIMENTAL

*Methyl Celastrol.*—Because this derivative could be obtained in such an excellent crystalline state and because it appeared to have no solvent of crystallization, its ultimate analysis was highly desirable.

Analysis:

|                   |            |                            |                       |
|-------------------|------------|----------------------------|-----------------------|
| $C_{23}H_{32}O_3$ | Calculated | C 77.56                    | H 8.99                |
|                   | Found      | C 77.67,<br>77.83<br>77.51 | H 8.97, 9.13,<br>8.96 |

*Celastrol.*—The values for carbon and hydrogen reported in the previous paper would not agree with the formula  $C_{22}H_{30}O_3$ .

It had been noted that celastrol appeared to undergo decomposition at the melting point with the evolution of a gas. In order to determine the nature of this gas, an experiment was devised whereby dry nitrogen was passed over a sample of celastrol (previously dried at  $100^\circ C.$  in a vacuum) at the melting point. The gases were then passed through dryrite and ascarite in order to absorb any water or carbon dioxide that might be evolved. No carbon dioxide or water was given off but the original compound lost weight. This was calculated as solvent (isopropyl ether) still retained by celastrol. A 0.2034-Gm. sample lost 0.0144 Gm., or 7.08 per cent, equal to one-fourth molecule of isopropyl ether. Therefore one molecule of isopropyl ether was present to four molecules of celastrol.

Analysis:

|                                     |            |                   |                 |
|-------------------------------------|------------|-------------------|-----------------|
| $(C_{22}H_{30}O_3)_4 \cdot C_3H_8O$ | Calculated | C 76.73           | H 9.11          |
|                                     | Found      | C 76.66,<br>76.59 | H 9.08,<br>8.65 |

*Reduction of Celastrol.*—A qualitative catalytic reduction of celastrol by means of platinum black at room temperature and 40-lbs. pressure produced a colorless solution. The catalyst was removed by filtration and before it could be completed the solution became colored yellow and upon standing soon turned orange-red.

*Reduction of Methyl Celastrol.*—The reduction was carried out in the same manner described above. The same phenomenon took place. A portion of the colorless solution was tested with ferric chloride before oxidation could take place and an intense green color developed instantly.

*Oxidation of Methyl Celastrol with Hydrogen Peroxide.*—One-half gram of methyl celastrol was dissolved in an excess of alcoholic potassium hydroxide. Hydrogen peroxide (30%) was added until the deep red color was completely destroyed. This reaction took place very rapidly. The reaction mixture was acidulated with phosphoric acid and extracted with ether. The ethereal solution in turn was extracted with aqueous sodium bicarbonate. The combined bicarbonate extracts were washed once with ether, decomposed with phosphoric acid and extracted with ether. The ethereal extract was washed with water and the ether concentrated. About one-fourth Gm. of oxidized product was obtained which corresponded to one-half of the starting material. This oxidized material has not been obtained in a crystalline form as yet, but it appears to be acidic.

*Reduction of Celastrol and Methyl Celastrol with Sulfurous Acid.*—When an alcoholic solution of celastrol was treated with sulfurous acid the mixture immediately became colorless. Upon removal of the sulfurous acid whether by aeration or neutralization with alkali, the solution become colored again. Ortho quinones can be reduced by sulfurous acid.

When methyl celastrol was treated in the same fashion, the same phenomenon was observed.

*Saponification of Acetyl Methyl Celastrol.*—When potassium hydroxide was added to an alcoholic solution of acetyl methyl celastrol, the solution immediately turned dark red. If sodium carbonate was substituted for potassium hydroxide no immediate red color appeared. However, a red color soon developed which increased in intensity from a very pale red to a deep garnet-red. The mixture was then diluted with water and extracted with ether. The ether was removed on the steam-bath and the residue taken up in alcohol. The alcoholic solution gave a green color with ferric chloride T.S.

This experiment indicates that the acetyl groups are extremely sensitive to alkaline hydrolysis.

*Methylation of Methyl Celastrol by Dimethyl Sulfate and Alkali.*—Methyl celastrol was methylated in the usual manner with dimethyl sulfate and alkali. Because methyl celastrol and celastrol are unstable in the presence of alkali, it is doubtful if a uniform product was obtained. This methylated product was yellow and could not be crystallized. The methoxyl content indicated the equivalent of almost two methoxyl groups.

Analysis—OCH<sub>3</sub>:

C<sub>24</sub>H<sub>34</sub>O<sub>3</sub> Calculated for 2 methoxyl groups 16.75 per cent  
 Found 16.15, 16.00; av. 16.08 per cent

*Acetyl Value of Acetyl Celastral.*—Acetyl celastral was saponified with an excess of alcoholic alkali. The reaction mixture was acidulated with sulfuric acid and the liberated acetic acid distilled into standard alkali.

Analysis—CH<sub>3</sub>CO:

C<sub>26</sub>H<sub>34</sub>O<sub>5</sub> Calculated for 2 acetyl groups 20.14 per cent  
 Found 21.44, 21.75; av. 21.59 per cent

*Acetyl Value of Acetylated Methylated Celastral.*—This analysis was carried out like the above analysis.

Analysis—CH<sub>3</sub>CO:

C<sub>27</sub>H<sub>36</sub>O<sub>5</sub> Calculated for 2 acetyl groups 19.50 per cent  
 Found 19.82, 20.69; av. 20.25 per cent

*Attempted Reduction of Methyl Celastral with Aluminum Isopropylate.*—Aluminum isopropylate was prepared according to the method of Homer Adkins (2). A test reduction of methyl celastral with aluminum isopropylate indicated that an aluminum salt of methyl celastral was formed, because the mixture assumed a deep red color. The mixture when heated for ten hours did not change in color intensity and no reduction took place. According to Lund (3), ketones which tend to enolize and form aluminum salts cannot be reduced by this method.

## SUMMARY

It has been shown that celastral, a red pigment found in the outer bark of the root of *Celastrus scandens*, has the formula C<sub>22</sub>H<sub>30</sub>O<sub>3</sub>. The molecule contains an hydroxyl group sufficiently acidic enough to be methylated by means of diazomethane. It appears that the remaining two oxygen atoms are present in the form of an ortho quinone which can be reduced catalytically and reoxidized by atmospheric oxygen.

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## Water in Oil Emulsifying Agents. I\*

## Some Constituents of Lanolin and Similar Compounds

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Water-in-oil emulsifying agents are used extensively in the manufacture of ointments and cosmetics. Among the more widely used emulsifying agents for these purposes are waxes such as lanolin spermaceti, and beeswax; polycyclic alcohols such as sterols; and aliphatic alcohols of high molecular weight, some of which are components of certain waxes. Of these, lanolin and waxes were used by the ancients (1). More recently ointment bases of high water absorptive power have been introduced, and are usually described in rather vague terms as consisting of certain alcoholic components of lanolin combined with petrolatum (2). Several patents have been issued on methods of obtaining components of lanolin with unusual hydrophylic properties, of which those granted to Lifschütz (3), (4), Frick (5) and Barnes (6) are examples. Cholesterol has been recommended frequently as a useful adjunct to petrolatum for increasing its ability to absorb water, and for enhancing its value from a therapeutic or cosmetic standpoint (7), (8), (9), (10), (11), (12), (13). Blatz (14), some years ago, showed that cetyl alcohol when mixed with petrolatum produced an ointment base capable of absorbing 60% to 70% of water.

The earlier investigations of the chemical components of lanolin by Schulze (15), (16) and by Darmstaedter and Lifschütz (17) indicated that the unsaponifiable portion consisted principally of cholesterol, iso-cholesterol and aliphatic alcohols such as ceryl alcohol. More recently iso-cholesterol has been resolved into agnosterol (18), C<sub>30</sub>H<sub>48</sub>O, and lanosterol (19), C<sub>30</sub>H<sub>50</sub>O. The investigations of Schulze (20) and others (21), (22)

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