

Analysis—OCH₃:

C₂₄H₃₄O₃ Calculated for 2 methoxyl groups 16.75 per cent
Found 16.15, 16.00; av. 16.08 per cent

Acetyl Value of Acetyl Celastrol.—Acetyl celastrol 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—CH₃CO:

C₂₆H₃₄O₅ Calculated for 2 acetyl groups 20.14 per cent
Found 21.44, 21.75; av. 21.59 per cent

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

Analysis—CH₃CO:

C₂₇H₃₆O₅ Calculated for 2 acetyl groups 19.50 per cent
Found 19.82, 20.69; av. 20.25 per cent

Attempted Reduction of Methyl Celastrol with Aluminum Isopropylate.—Aluminum isopropylate was prepared according to the method of Homer Adkins (2). A test reduction of methyl celastrol with aluminum isopropylate indicated that an aluminum salt of methyl celastrol 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 celastrol, a red pigment found in the outer bark of the root of *Celastrus scandens*, has the formula C₂₂H₃₀O₃. 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), C₃₀H₄₈O, and lanosterol (19), C₃₀H₅₀O. The investigations of Schulze (20) and others (21), (22)

* Presented before the Scientific Section, AMERICAN PHARMACEUTICAL ASSOCIATION, Atlanta meeting, 1939.

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indicate that neither agnosterol nor lanosterol is a true sterol. Sobotka (23) states, "They have thirty carbon atoms and a picene skeleton, consisting of five six-membered rings with six non-nuclear methyl groups. On dehydrogenation, they never yield methyl-CPP,¹ but picene and a number of substituted naphthalenes."

Lifschütz (24) has shown, in a semi-quantitative way, the influence of certain alcoholic components of lanolin as water-in-oil emulsifying agents when used with petrolatum. Lifschütz (25) has also pointed out that lanolin as an ointment base possesses chemical and physical disadvantages, and that its water-absorptive property is due to the presence of from 5% to 8% of free alcohols. He recommends a mixture of 3% of these alcohols with petrolatum as an ointment base which will absorb from 300% to 400% of water. Casparis and Meyer (26) have determined the maximum amount of water which mixtures of several different waxes and alcohols with petrolatum and lard are capable of absorbing. The maximum quantity of water capable of being held at ordinary temperatures (20° C.) by 100 Gm. of water-free ointment base, they designate as "water number."

Despite the extensive literature relating to lanolin and its use and the use of its components in hydrophylic ointment bases, some confusion continues to exist. Accordingly, an investigation was undertaken to ascertain with some degree of certainty what components of lanolin are responsible for its ability to absorb water, and, if possible, to develop an easily obtainable hydrophylic emulsifier which when mixed with petrolatum would produce a water-absorbent ointment base for general use, but without the inherent objectionable features of lanolin when used alone.

Anhydrous lanolin was saponified by three different methods: (1) that of Doree and Garratt (19) involving the use of sodium ethylate in anhydrous ether; (2) heating the lanolin with alcoholic potassium hydroxide under a reflux condenser during twelve hours; and (3) by a cold method not previ-

ously described for the saponification of lanolin. The latter method appeared to be the most convenient and quite satisfactory since it yielded as good results as the first method. The unsaponifiable portion was separated from the soaps and resolved into four fractions, the first, consisting of a mixture of aliphatic alcohols, the second, of the so-called "iso-cholesterol," the third, of cholesterol, while the fourth fraction was an amorphous mixture from which no crystalline material could be isolated. The partial separation of the unsaponifiable portion was accomplished by modifications of the methods described by Doree and Garratt (19), Heiduschka and Nier (27) and Kuwata and Katuno (28). Mixed amyryns were obtained from Manila Elemi Gum, and resolved into α - and β -forms through the benzoates by the method of Horrmann (29). Cholesterol esters of lauric, palmitic and stearic acids were prepared by the procedures described by Page and Rudy (30). The cetyl alcohol used was obtained from the Eastman Kodak Company. The water-absorbent powers of these various substances in different concentrations in white petrolatum² were determined. Amyrin was included in the list because of the possibility that it might develop that there is a close chemical relationship between it and "iso-cholesterol."

EXPERIMENTAL

Saponification of Lanolin.—To 800 Gm. of melted anhydrous lanolin (Merck) were added 200 cc. of alcohol, and the mixture was cooled to 34°. To this mixture were added with vigorous stirring 270 Gm. of potassium hydroxide dissolved in 300 cc. of water, and cooled to room temperature. When the reaction had subsided, the mixture was placed in an oven at 50°, and dried for twenty-four hours. It was then mixed with 600 Gm. of anhydrous sodium sulfate, transferred to a Soxhlet extractor, and extracted with acetone until all of the unsaponifiable portion and a small amount of the soap were removed. The acetone solution was concentrated to a volume of 500 cc., one L. of ether was added, and the solution was washed with water in a separatory

² The white petrolatum which appeared to give best results was White Protopet No. 1 produced by L. Sonneborn and Sons. We wish to thank Dr. Erich Meyer for suggesting its use, and for supplying us with an adequate quantity for our experiments.

¹ Cyclo-penteno-phenanthrene.

funnel several times. In this way all soaps were removed without the formation of an emulsion. Upon evaporation of the solvent, a residue weighing 300 Gm. was obtained.

Resolution of the Unsaponifiable Portion.—One hundred Gm. of the unsaponifiable portion of lanolin were dissolved in hot acetone. The solution, upon cooling, deposited 17 Gm. of nearly white amorphous material which after one recrystallization from methyl alcohol, followed by one recrystallization from acetone, yielded 9 Gm. of crystalline material melting at 58–59°, with preliminary softening. On the basis of work previously referred to (19), (27), (28), this was considered to consist of a mixture of aliphatic alcohols. No attempt to make a further separation of this first fraction was successful.

The acetone (675 cc.) was removed by distillation from the solution remaining after the separation of the mixture of aliphatic alcohols. The residue was extracted repeatedly with hot methyl alcohol until a white, pulverulent residue weighing 9.5 Gm. remained. This material, after one recrystallization from a mixture of acetone and methyl alcohol, and drying at 100°, melted at 135–136° with preliminary softening. This is near the melting point given in the literature for iso-cholesterol, and since no precipitation was produced from an alcoholic solution of this second fraction when an alcoholic solution of digitonin was added, it was considered to be sufficiently pure for testing its emulsifying power for water when mixed with petrolatum.

The methyl alcoholic solution obtained from the preceding extraction was cooled to 15°, whereupon 18 Gm. of nearly white, crystalline material were obtained: This was recrystallized once from methyl alcohol, and then acetylated by means of acetic anhydride. The mixture of acetates, recrystallized several times from 95% alcohol, and finally from acetone, yielded a small fraction (2.2 Gm.) melting at 112–114°. When this was mixed with cholesteryl acetate, there was no depression in melting point. Upon saponification, and recrystallization of the product from 95% alcohol, cholesterol melting at 146–147° was obtained. Mixed m. p., 145–146°. Since appreciable quantities of pure cholesterol could not be obtained from this third portion by fractional crystallization, commercial cholesterol (Eastman's) was used in the water absorption experiments.

The methyl alcoholic solution, after removal of the 18 Gm. of crystalline material, was concentrated in volume, and an additional 20 Gm. of yellowish white, amorphous material was obtained. This, after recrystallization from acetone, melted at 55–60°. No attempt to make a further separation by fractional crystallization was successful. Finally, upon removal of all of the methyl alcohol, 33 Gm. of an amorphous, gummy residue remained. No crystalline material could be obtained from this fourth fraction, and it remained apparently unaltered when boiled during several hours with 15% alcoholic potassium hydroxide.

Preparation of Cholesteryl Laurate.—Four Gm. of cholesterol (Eastman's, m. p. 145–146°) and 8 Gm. of lauric acid (Eastman's, m. p. 42–43°) were thoroughly mixed. The mixture was heated during 3 hours in an atmosphere of carbon dioxide at a temperature of 200°. The product, recrystallized three times from alcohol, yielded 5.5 Gm. of the ester melting at 91° (30).

Preparation of Cholesteryl Palmitate.—Five Gm. of cholesterol and 6 Gm. of palmitic acid (Eastman's, m. p. 61–62°) were thoroughly mixed, and then heated during two hours in an atmosphere of carbon dioxide at a temperature of 210–220°. After recrystallizing the product four times from alcohol, 6 Gm. of the ester melting at 89° with preliminary softening were obtained (30).

TABULATION OF EMULSIFYING POWER OF SOME WATER IN OIL EMULSIFYING AGENTS WITH PETROLATUM

Emulsifying Agent in White Petrolatum	Per Cent of Water Emulsified
Cholesterol 3%	250%
Cholesterol 3% + Cholesteryl Acetate 3% ^a	500%
Cholesterol 3% + Cholesteryl Laurate 3% ^a	600%
Cholesterol 3% + Cholesteryl Palmitate 3% ^a	700%
Cholesterol 3% + Cholesteryl Stearate 3% ^a	800%
Cholesterol 3% + Spermaceti 3%	500%
Cholesterol 3% + White Wax 3%	500%
Spermaceti 3%	100%
Iso-Cholesterol 3% ^b	300%
α-Amyrin 2% ^b	50%
β-Amyrin 2% ^b	50%
Mixture of Aliphatic Alcohols (Fract. I) 3% ^c	400%
Cetyl Alcohol 3% ^c	75%
Total Unsaponifiable from Lanolin 5%	350%
Unsaponifiable 4% + White Wax 4%	600%
Fract. 4 from Unsaponifiable 3%	400%

^a Cholesterol esters when used alone have no emulsifying action.

^b The efficiency of the amyriins and iso-cholesterol is not appreciably increased when combined with their esters or with waxes.

^c It is not possible to reconcile these different results unless it is assumed that they are due to the longer carbon chain in ceryl alcohol. The efficiency of cetyl alcohol is not materially increased by mixing it with small amounts of cholesterol or iso-cholesterol.

Preparation of Cholesteryl Stearate.—Four Gm. of cholesterol were thoroughly mixed with 6 Gm. of stearic acid (Eastman's, m. p. 69–70°). The mixture was heated during three hours in an atmosphere

of carbon dioxide at a temperature of 210–220°. The product was recrystallized twice from a mixture of equal volumes of acetone and methyl ethyl ketone. It then was dissolved in 50 cc. of ether, and precipitated by the addition of 200 cc. of alcohol. Yield, 6.1 Gm. Melting point, 82° with preliminary softening (30).

Determination of Emulsifying Power.—Into each of five numbered 8" x 1" test-tubes were placed 5 Gm. of a 1% solution of cholesterol in white petrolatum. The first test-tube was immersed in a bath of boiling water, and heated until the solution reached a temperature of 65–70°. To this were added all at one time, with mechanical stirring by means of a spiral glass stirrer reaching to the bottom of the test-tube, 2.5 cc. of water at the same temperature. The mixture was stirred until it had cooled to room temperature. Under identical conditions, 5 cc. of water were added to the second test-tube, 7.5 cc. to the third, 10 cc. to the fourth and 12.5 cc. to the fifth. Similarly, varying proportions of water were added to 2, 3, 4 and 5% solutions of cholesterol in white petrolatum. The emulsions thus formed were set aside for two months, and then examined. A decision was then reached as to the optimum concentration of cholesterol in petrolatum for the production of an emulsion containing a maximum amount of water. By identical methods the other emulsifying agents were similarly studied. In the tabulation which follows are reported the concentration and composition of the various emulsifying agents which were believed to be best for producing stable emulsions containing the highest concentrations of water.

SUMMARY AND CONCLUSIONS

1. Lanolin has been saponified and the unsaponifiable portion has been separated into four fractions.
2. The relative efficiencies of certain components of lanolin and some analogous compounds as water-in-oil-emulsifying agents with petrolatum have been determined.
3. It has been shown that the emulsifying efficiency of cholesterol is much less than the emulsifying efficiency of mixtures of cholesterol and cholesterol esters when used with petrolatum for the purpose of emulsifying water.
4. Any one combination of petrolatum and water-in-oil emulsifying agent as a suitable ointment base cannot be recommended until after further study.

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