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INVESTIGATION OF A BY-PRODUCT OF EPHEDRINE PROCESSING BY MARKER ALKALOIDS, QUETTA

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Introduction

Ephedra vulgaris Rich. out of which ephedrine was first isolated by Nagai,¹ and other species of the family *Ephedraceae* are usually extracted with benzene-petrolether mixtures after being made alkaline. After removing the solvent the residue is treated with hydrochloric acid so that all basic substances are eliminated. A grease-like, blackish-green material is left in quantities which justify a proper investigation of the material with a view to its further industrial utilisation.

All the sterols present in the plant must be concentrated in the above-mentioned greasy substance. A preliminary test actually showed that there is at least one sterol which gives a positive digitonin test wherefore it seemed worthwhile to study if the ephedra sterols could prove useful as a starting material for a cortizone I synthesis.

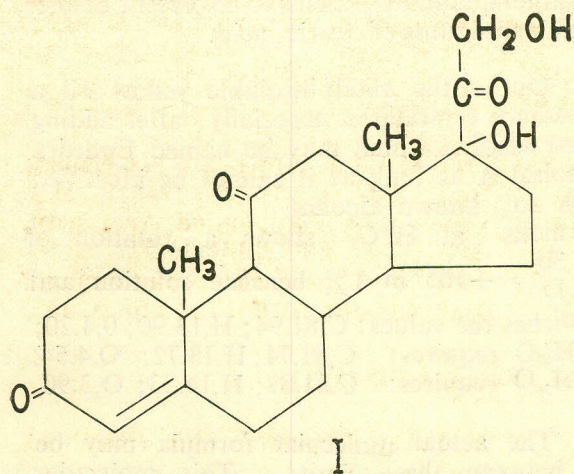
diosgenin from a Mexican yam and hecogenin present in the juices of the agave group of plants. Similarly the sisal also contains it and according to New Zealand Science Report of Jan. 1959 this source is already being used with success in the Commonwealth countries.

However, in 1956 two saponins—correllogenin and gentrogenin—were isolated in Mexico from sweet potatoes from which also a short process leads to cortizone.

The results of the present paper are given in Fig. I.

The grease contains 10% moisture which can be removed after 6 hours at 100°C. over phosphorpentoxide. Another 8% of the matter insoluble in organic solvents like benzene does not burn and is therefore considered to be dust.

The best method for further processing of the grease so far developed is its saponification, whereby it splits off in a saponifiable and an unsaponifiable part. Using a simple method the petrol-ether solution of the alkali insoluble oil can easily be separated from the heavily frothing soap solution. The soap after acidification can be divided into a soluble and an insoluble part of petrol ether. The soluble part contains fatty acids which represent 36% of the total grease. The insoluble part, a blackish mass, could be divided into a benzene soluble and an insoluble part. The benzene soluble part contains a glycoside, chlorophyll, carotin and a gum, the presence of them being still under investigation, while the insoluble part furnishes out of its ethyl acetate solution a black glossy laquer which being not at all



Previously this important hormone has been synthesised from cholic and desoxycholic acid in thirty stages. Later on short processes were used starting with ergosterol, stigmasterol,

Investigation of a grease-like by-product of the processing of Ephedra species
in Quetta Ephedrine Factory
(Marker Alkaloids)

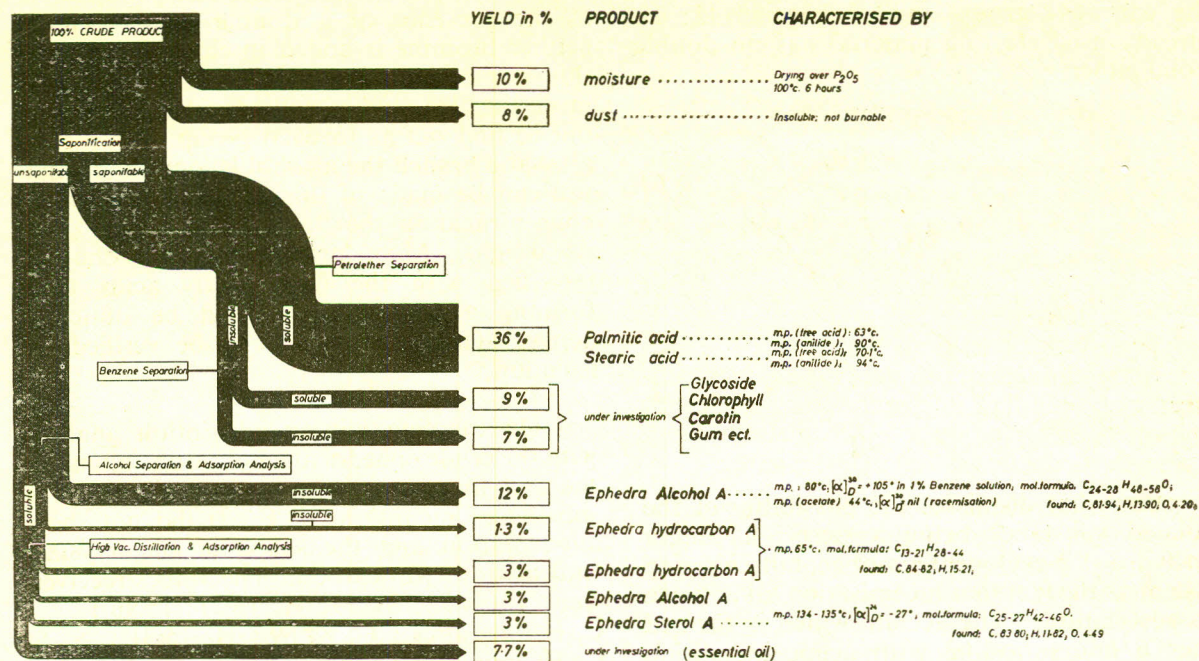


FIG 1.

sticky but very elastic may find use as water-proof covering thimble for bottles or something like that.

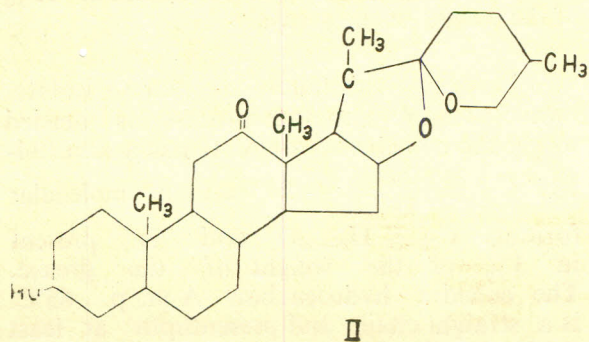
To spot out the fatty acids present was not easy. The iodine number of 37.2 excluded the presence of appreciable amounts of unsaturated ones. Purification through the urea inclusion compounds furnished an acid melting unsharp at 63°C. This indicates palmitic or stearic acid because the next lower acid the myristic melts at 54°C., while palmitic melts at 63.1°C. and stearic at 70.1°C. To further identify the acids they were converted into nicely crystalline anilides via the mixed anhydrides of phenyl carbamic and the fatty acids by reaction with phenyl isocyanate. Simply recrystallising these mixed anilides from alcohol did not have any success as regards their separation into the constituents present. Adsorption analysis on alumina, however, showed fairly reliably that only palmitic—

in larger quantities—seems to be present besides smaller amounts of stearic acid.

Out of the alkali insoluble yellow oil a substance crystallises, especially after adding some alcohol which may be named Ephedra Alcohol A as long as it cannot be identified with any known alcohol. It melts at 80°C, shows a rotation of $[\alpha]_D^{30} = +105^\circ$ in 1% benzene solution and furnishes the values: C, 81.94; H, 13.90; O, 4.20; C₂₄H₄₈O requires: C, 81.74; H, 13.72; O, 4.54; C₂₈H₅₈O requires: C, 81.87; H, 14.23; O, 3.90.

The actual molecular formula may be in between these limits. This molecular formula could indicate a sterol. However, the Liebermann-Salkowski test was negative. It follows that there is no double bond in the 5-6 position. Also there is no precipitate with digitonin so that there cannot be a

hydroxyl group in the 3- β -position. Both facts would not necessarily exclude the substance from being a starting material for a cortizone synthesis, because a 3- α -hydroxyl compound on oxidation would also furnish the carbonyl group and hecogenin II; an already used starting material has no double bond either.



But what dismisses the possibility of the isolated substance being a sterol, having a perhydro-1,2-cyclopenteno-phenanthrene ring system, is the fact that racemisation takes place on acetylation. The acetate which is formed after 6 hours boiling with acetic anhydride melts at 44°C. and shows no more rotation. To make sure that nothing else happened, the acetyl group was split off by boiling it with alcoholic hydrogen chloride. The deacetylated product had the same melting point of 80°C., the same solubilities and shape of the crystals as the active starting material and must, therefore, be considered to be the racemate. It follows that the hydroxyl group must be attached with the asymmetric C-atom and that there cannot be any other asymmetric centre at all. This is not the case with the structure of any sterol. The sterol ring system contains mostly four or even five asymmetric C-atoms, in any case always more than one. Even when racemisation would happen at one of the centres, the others must still retain their activity. Because of the above fact sterol structure for this substance can be excluded.

Boiling with alcoholic urea solution, however, furnished no sign of the formation of

an urea inclusion compound. This alcohol therefore, cannot have a straight chain. The data of the analysis agree better with $C_nH_{2n}O$ and $C_nH_{2n+2}O$ rather than with $C_nH_{2n-2}O$. In accordance with that there is no indication of the presence of a double bond because neither bromine is added in chloroform solution nor permanganate decolourised. Some more detailed studies will have to be carried out to find out as to what group of cyclic or aliphatic branch the alcohol belongs and what use can be made of this material which is concentrated in the "grease" up to 15% of its weight. Most probably this alcohol is esterified with the found fatty acids thus forming a wax which should be optically active. This however has to be verified by isolation.

On carrying out an adsorption analysis with the crude ephedra alcohol A on alumina, the first five fractions out of 66, furnished a substance melting at 65°C. It did not show any rotation and the shape of the crystals indicated a hydrocarbon. The values received were: C, 84.82; 84.59; H, 15.21; 15.96; $C_{13}H_{28}$ requires; C, 84.69; H, 15.31; $C_{31}H_{64}$ requires; C, 85.23; H, 14.77. The actual molecular formula lies in between these limits.

With urea, an inclusion compound is formed in alcoholic solution. It follows that the hydrocarbon must have an unbranched chain. While bromine in chloroform solution did not have any effect on it and neutral permanganate was not decolourised, the latter happened immediately in alkaline medium. The total amount isolated from the grease comes to 4.3 %.

After removing all the material which crystallises under conditions used the yellow oil residue was submitted to high vacuum distillation. The wide range within which it boils at 0.06 mm, namely 115-265°C. indicates its complex character. It was divided arbitrarily into five fractions. The first boiling from 115-150°C consisted only of essential oil. Out of the second and third some more hydrocarbon A and ephedra

alcohol A could be isolated, while fraction IV furnished a real sterol named ephedra sterol A. Its melting point is 134-135°C. (from ethylacetate) and its $[\alpha]_D^{29} = -27^\circ$ in 1% benzene solution. The analysis gave the following values:

C, 83.8; H, 11.82; O, 4.49;

$C_{23}H_{38}O$ requires: C, 83.57; H, 11.57; O, 4.84;

$C_{27}H_{46}O$ requires: C, 83.87; H, 11.99; O, 4.14;

The real molecular formula may be in between these limits.

The sterol gives a precipitate with digitonin as well as a positive result with Liebermann test. In agreement with that it decolourises bromine in chloroform solution as well as permanganate in alkaline and acidic medium. On acetylation no racemisation takes place. The acetyl derivative melts at 118-120°C. and has still $[\alpha]_D^{29} = -39^\circ$ in 1% benzene solution.

Its amount totals up to at least 3% of the grease. Having at least a 3- β -hydroxyl group and a double bond in 5-6 position it has some elements useful for a cortizone synthesis, but lacking any other oxygen-containing group it offers no advantages compared with the already mentioned starting materials.

Fraction V was also found to contain a small amount of sterol A. In spite of a very thorough investigation of all constituents of the grease no other substances besides the essential oil, which will have to be further investigated, could be isolated.

This fact could be confirmed also by an adsorption analysis carried out with the crude grease itself. Only these three substances could be found besides the fat, or wax, chlorophyll, carotin, the glycoside and the essential oil, by thoroughly studying 276 fractions into which the grease was split off.

Summary

The investigation of the "grease", a by-product of ephedrine processing at Marker

Alkaloids, Quetta revealed that it contains fatty acids 36% of its weight, most probably a lesser proportion of stearic besides a larger amount of palmitic acid. Further, there is a high molecular alcohol named ephedra-Alcohol A m.p. 80°C. $[\alpha]_D^{30} = +105^\circ$ of the molecular formula $C_{24-28}H_{48-58}O$ present in about 15% of the weight of the grease.

These two substances are easy to isolate, while a more elaborate process is needed to get the ephedra sterol A. It has a m.p. 134-135°C., $[\alpha]_D^{24} = -27^\circ$ with a molecular formula $C_{23-27}H_{38-46}O$ and is present in 3% of the weight of the grease. The ephedra hydrocarbon A m.p. 65°C. is a straight chain and present up to at least 4.3%. Further, there is an essential oil in an amount of approximately 7% which however has neither a particular smell which might make it useful as a perfume, or could make it of any other industrial importance. Its investigation—high vacuum distillation—will be continued.

Finally, there is a glycoside present mixed with gum, chlorophyll, carotin and other substances, which seems easily separable from accompanying substances of similar solubilities. Efforts in this direction will be continued.

Acknowledgement

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References

1. Nagai, Pharm. Zfg., 32. 700 (1887)

EXPERIMENTAL

1. Preliminary tests.—The by-product in the processing of ephedrine the so-called "grease" is a blackish green wax-like material with approximately 10% moisture which it loses on drying over phosphor pentoxide at 100°C. during 6 hours. On dissolving that grease in organic solvents like benzene, 8% of it by weight remain undissolved and is considered to be dust. On steam distillation with 20 g. nothing volatile is visible only a menthol-like smell is noticed. The water above the residual grease shows a p_H of 4.8.

2. Chromatography of the raw grease on alumina.—15 g. of the raw grease were dissolved in 100 ml. of hot benzene and filtered from 1.2 g of the residue. Considering that 10% moisture is equal to 1.5 g this solution contains 12.3 g of the substance. It was passed through a column of 450 g. of alumina (Brockmann, E. Merck) of 3.5 cm width and 60 cm length, then benzene, ethyl acetate and finally alcohol was taken as the eluent.

With benzene the grease displayed different colours from dark green at the top to yellow, light green, brown, pinkish red and finally again light green. 276 fractions were obtained. According to microscopic and solubility tests the several fractions were combined to twelve groups as given in Table 1:—

Table 1

Fractions	Groups	Amount
1— 14	.. I	3.30 g.
15— 26	.. II	1.663 g.
27— 57	.. III	0.677 g.
58— 79	.. IV	0.342 g.
80—105	.. V	0.110 g.
106—130	.. VI	0.107 g.
131—198	.. VII	0.206 g.

199—241	.. VIII	0.75 g.
242—251	.. IX	1.115 g.
252—262	.. X	0.181 g.
263—269	.. XI	0.5365
270—276	.. XII	1.6755
		recovered : 10.6634 g.
		moisture : 1.5
		dust : 1.2
		13.3634 g.
		loss : 1.6456 g.
		15.00 g.

Investigation of Group I.—This group consists of a pale waxy mass mixed with an oil. All efforts to get the wax in a crystalline form failed. Also a new absorption analysis on alumina collecting 33 fractions using petrol ether, benzene and finally chloroform as eluents did not bring a separation. The mixture was spread throughout these fractions.

Investigation of Group II.—Same oily wax as I with some crystals. 1.663 g. of the group on treatment with benzene gave 1.14 g. of a crystalline substance which on further crystallisation melted at 80°C. It is identical with Ephedra alcohol A isolated in larger quantities on the saponification described in experiment 4a.

Investigation of Group III.—It is a waxy green material with some crystals in it. All attempts to get these crystals by recrystallisation failed. Therefore, the whole (0.677 g.) was dissolved in acetone and passed through a small column of alumina (10 g.) using acetone as eluent. The only substance which could be obtained by that means was again Ephedra-Alcohol A. m.p. 78-80°C. 0.431 g.

Investigation of Group IV.—This is also a green wax-like mass (0.342 g.) with some crystals. The whole was dissolved in 20 ml.

of ethyl acetate, filtered and concentrated to 10 ml. and kept standing. Received: 0.146 g., m.p. 76-78°C. After further recrystallisation in acetone the m.p. kept constant at 78-80°C. This compound is also identical with Ephedra-Alcohol A.

Investigation of Group V.—Its appearance is the same as IV. By treating this material with acetone a few needles could be isolated which melted at 130-131°C. They are obviously identical with ephedra sterol A isolated in larger quantities in experiment 4c.

Investigation of Group VI.—This group (0.107 g) seems to contain wax with a different type of crystals in it. It was dissolved in ethyl acetate, filtered, somewhat concentrated and cooled, when 0.085 g of a colourless substance was received melting at 60-62° C.—obviously the ephedra hydrocarbon A as seen in experiment 4b.

Investigation of Group VII.—This group (0.206 g) is nearly a colourless waxy material. It was dissolved in 15 ml of ethyl acetate, filtered, concentrated to approximately 10 ml. and cooled. After standing for sometime, 0.075 g. of colourless material were obtained melting at 58-60° C., which were identical with the hydrocarbon A.

Investigation of Group VIII.—This yellowish waxy material (0.75 g) was dissolved in ethyl acetate, filtered, concentrated and kept standing. 0.43 g. of crystalline substance came out melting at 60-65°C (not sharp) which was probably a mixture of hydrocarbon with Ephedra alcohol A.

Investigation of Group IX.—1.115 g. of the material when dissolved in ethyl acetate gave colourless crystals melting at 78-80°C.

Investigation of the Groups X-XII.—Out of these dark green ethyl acetate solutions nothing crystalline could be isolated.

3. Saponification of the raw grease.—200g. of potassium hydroxide pellets were dissolved in 800 ml. of ethanol, added to 1 kg. of the raw grease in a 4 l. round bottom flask and refluxed in water bath for 16 hours. The alcohol

was then removed in vacuum and the residue diluted with 12.5 l. of water. To remove the alkali insoluble from the heavily frothing soap solution the following procedure was found useful.

In portions of 2 l. each, the saponified mixture was shaken with 500 ml. of petrol-ether and kept standing either in refrigerator for 2 hours or at room temperature overnight. Thus a clear separation was achieved. By repeating this manipulation, the alkali insoluble material, a yellow oily mass, could be satisfactorily removed from the soap solution.

After washing the petrolether solution with water, drying it over sodium sulphate, the petrolether was removed, some alcohol added and the already crystallising mass kept standing overnight.

(i) crop of yellowish crystals: 118.0 g.

(ii) crop from the mother liquor after some days: 14.5 g.

The total of 132.5 g. melted (unsharp) at 67° C.

When no more crystals came out, the alcohol was removed. The residue of honey like oily mass weighed 109.2 g.

The soap solution was acidified with concentrated hydrochloric acid and the out-coming fatty acids taken up in petrolether. A blackish pasty mass remained undissolved. The petrolether solution was washed with water, dried over sodium sulphate and freed from the solvent. Yield: 368.6 g.

The pasty mass was mechanically collected, dried on water bath to constancy: 158 g.

Balance: alkali insoluble crystals	..	132.5 g.
" oil	..	109.2 g.
" pasty mass	..	158.0 g.
" fatty acids	..	368.6 g.
		<hr/>
Total	..	768.3 g.
dust	..	80.0 g.
moisture	..	100.0 g.
		<hr/>
		948.3g.

Investigation of the alkali insoluble part;—
Isolation of Ephedra Alcohol A. 30 g of the yellow crystalline material melting unsharp at 67°C., were dissolved in 150 ml of petrol ether on warming and given on top of a column of alumina (Brockmann, Merck) of 120 cm. length and 3.5 cm breadth. Petrol-ether was used as eluent. The column consisted of two tubes of 60 cm. length each connected by interchangeable ground glass joints. After collecting 66 fractions containing 19.5312 g., the column was broken in two parts of 60 cm each in order to accelerate the elution. The lower column was further eluted with petrol ether furnishing 2.2610 g. of the substance in 7 more fractions. The upper part was treated with chloroform, whereby in 22 fractions 8.2470 g were brought out of the alumina, thus bringing the balance to 30.0392 g.

The above breaking up of the long column could be done because the investigation of several fractions revealed that only the first five fractions contained a different kind of crystals, while the whole of the remaining fractions, No. 6-66, furnished only one shape of crystals melting at 78-80°C. The same was found after dismantling the long column into its upper and lower parts. Out of both parts the only crystalline substance which could be obtained melted at 78-80°C. After repeated recrystallisations from benzene the melting point kept constant at 80°C.

$[\alpha]_D^{30} = +105^\circ$ in 1% benzene solution.

found: C, 81.94; H, 13.90; O, 4.20 ;
 $C_{24}H_{48}O$ (352,62) requires: C, 81,74; H, 13,72;
 O, 4.54
 $C_{28}H_{58}O$ (410,74) requires: C, 81,87; H, 14,23;
 O, 3.90;

Within these two limits the real molecular formula must lie.

Liebermann-Burchardt test.—Some mg. of the substance were dissolved in chloroform and a mixture of acetic anhydride and conc. sulphuric acid added. No colouration took place. It follows that there is no double bond in position 5-6 of an eventually present setrol.

Digitonin test.—By mixing the somewhat warm solution of the substance in 90 % alcohol with the alcoholic solution of digitonin no precipitation occurred. Therefore, no 3- β -hydroxylgroup is present.

As the absence of a double bond has been indicated by a negative Liebermann test, permanganate was not decolourised nor bromine in chloroform solution added.

Acetyl-ephedra alcohol A.—2.5 g of the pure alcohol were refluxed for 6 hours with 30 ml. of acetic anhydride. On cooling colourless crystals came out which were filtered by suction and pressed on a porous plate and weighed 2.49 g. When recrystallised from acetone the melting point kept constant at 44°C. There was no more rotation in 1% benzene solution.

Found: O, 7.90; $COCH_3$, 10.4. $C_{28}H_{50}O_2$
 394,66) requires: O, 8.11;

To determine the acetyl content the substance was refluxed for two hours with alcoholic sodium hydroxide. Only 2.2% acetyl could be found. Therefore the deacetylation was done with alcoholic hydrochloric acid.

Deacetylation of Ephedra Alcohol A acetate.—1.465 g. of pure acetate were refluxed for 7 hours with 5% dry hydrogen chloride containing methanol. Cooled, filtered by suction and recrystallised from benzene. Yield: 1.4 g., m.p. 80°C. There was no more any rotation in 1% benzene solution.

4-b. Isolation of Ephedra-hydrocarbon A.

The total (2.329 g.) of the first five fractions of adsorption analysis described above were thrice recrystallised from ethyl acetate when the melting point kept constant at 60-62°C. However, only after further recrystallising *this material*, twice from acetone an oxygen containing contamination could be removed and the melting point raised upto 63-65°C. The analysis now totalled up to 100%.

Found: C, 84,82; 84.59; H, 15.21; 14.96

$C_{18}H_{28}$ requires C, 84.69; H, 15.31

$C_{31}H_{64}$ requires: C, 85.23; H, 14.77

Within this range may be the real molecular formula.

An urea inclusion compound could be found in alcoholic solution, wherefore an unbranched chain is present. Accordingly there was no optical activity in 1% benzene solution. While neutral permanganate solution did not affect the substance, there was an immediate decolourisation with alkaline

permanganate. Bromine in chloroform solution was kept standing with the substance for several hours but the unchanged starting material could be gained back.

4-c. Isolation of Ephedraesterol A.—7.0 g of the honey-like yellow oil which remained after the removal of the alcohol A and some of the hydrocarbon A with alcohol was subjected to high vacuum distillation using the copper block 2. Five fractions were collected according to Table 2.

TABLE 2

Fraction No.	Temp	Pressure	Amount	Remarks
I.	115-150°C.	0.07 mm.	1,049 g.	Temp. rises constantly. The distillate remains oily.
II.	151-180°C.	„	1,632 g.	The distillate solidifies. There is a ha round about 160°C.
III.	181-188°C.	„	0,777 g.	The dist. solidifies much quicker and is bright yellow.
IV.	189-238°C.	„	1,546 g.	There is a quick dist., which solidifies and is bright yellow.
V.	239-265°C.	„	0,694 g.	There is only a slow dist., which solidifie
		Total ..	5,698 g	
		Residue ..	0,984 g.	

TABLE 3.

Fract No.	Temp	Pressure	Amount	Remarks
1.	130-150°C	0.06 mm	4,801 g.	Yellow, clear oil.
2.	151-160°C	„	4,325 g.	Somewhat deeper yellow oil.
3.	161-170°C	„	2,121 g.	Same like 2.

Investigation of Fraction I.—This yellow oil which did not crystallise on standing does not have a strong but a characteristic smell. It was again submitted to high vacuum distillation according to Table 3 using larger amounts from several similar fractions.

The fraction 1 decolourises bromine solution in chloroform and also neutral permanganate solution. Its infrared spectrogram indicates the presence of a hydroxyl and a carbonyl group, and also of a double bond. The still wider range of the boiling points shows that this essential oil is a mixture of several constituents. It will be studied later on.

Investigation of fraction II.—26.04 g. of the collected material were dissolved in 50 ml. of ethyl acetate by heating. On cooling 2,104 g. of crystals came out melting at 64-65°. On repeated recrystallisation the m.p. rose up to 68-70°C., but the shape of the crystals was not uniform. Therefore the ethyl acetate solution was passed through a alumina column where it could be separated into ephedra alcohol A melting at 78-80°C, and a small amount of the hydrocarbon A m.p. 65°C.

Investigation of Fraction III.—3.0 g of the fraction distilling between 181-188°C. 0.07 mm were dissolved in 100 ml. of warm acetone. On cooling 1.315 g. of a crystalline substance melting at 66-68°C. were received. After three more recrystallisations the melting point rose up to 68-70°C. but the microscopic picture showed still a mixture of different shapes. Using again an alumina column with a mixture of chloroform-ethylacetate (1 : 3) as eluate, pure Alcohol A could be isolated. The mother liquor consisted merely of the essential oil. Hydrocarbon was no more detectable.

Investigation of fraction IV.—2.5 g. distilling between 189-238°C. were dissolved in 35 ml of acetone by heating and kept standing for crystallisation. 1.10 g. melting at 58-62°C. could be gained. Repeated recrystallisations from acetone identified this substance to be the hydrocarbon A m.p. 65°C. The rest is essential oil.

Mother liquor on concentrating furnished a crop of crystals which after repeated recrystallisation from acetone melted at 134-135°C.

Much better is the separation when fraction IV is passed through an alumina column. The first three fractions eluted with acetone contain the hydrocarbon while the rest of the acetone elutes furnishes the new sterol A.

In 1% benzene solution it shows an $[\alpha]_D^{24} = -27^\circ$

found: C, 83.8; H, 11.82; O, 4.49

$C_{28}H_{38}O$ requires: C, 83.57; H, 11.57; O, 4.84

$C_{27}H_{46}O$ requires: C, 83.87; H, 11.99; O, 4.14

Within these two limits the real molecular formula might lie.

The Liebermann Salkowski test was found positive which indicates a double bond in the 5-position. Also with digitonin there was a precipitate. It follows that the one atom of oxygen present must belong to a hydroxyl group in 3- β -position.

In accordance herewith bromine is easily added in chloroform solution and permanganate decolourised in alkaline as well as in acidic medium. The hydroxyl group could, furthermore, be established by formation of an acetyl derivative.

Acetylation of Ephedra Sterol A.—200 mg. of the pure sterol A were refluxed in 3 ml. of acetic anhydride for 5 1/2 hours. On cooling crystals came out which were filtered by suction. Received: 185 mg m.p. 115-117°C. After three times recrystallisation from acetone the melting point kept constant at 118-120°C.

$[\alpha]_D^{29} = -39^\circ$ in 1% benzene solution.

Investigation of fraction V.—3.598 g. of this fraction were dissolved in acetone and passed through a column of 24 in. length containing 20 g. of alumina. 9 fractions were collected using acetone as eluent. While

the first and second fractions furnished only essential oil without any crystalline substance, the third and fourth fractions of 0.55 g. gave 0.16 g. of a solid substance melting at 60-62° C. being a mixture of a small amount of hydrocarbon and sterol A. The combined fractions 5-9 furnished from acetone 0.253 g., m.p. 133-134° C., and a second crop from the mother liquor also melting at 126-128° C. both undoubtedly sterol A. the rest is essential oil.

Out of the investigation of the five fractions it was found that besides essential oil there are hydrocarbon, the alcohol and sterol A.

5. Investigation of the saponifiable part of the grease.—The soap solution of experiment 3 had already been acidified and the outgoing fatty acid divided in 368.6 g. of petrol ether soluble fatty acids and 158 g. of a blackish petrolether insoluble mass. The raw fatty acids showed an iodine value of 37.2. It follows that there are practically no unsaturated fatty acids present.

Purification through urea inclusion compounds.—200 g. of the crude but dried petrol ether soluble acids were dissolved in 1 l. of methanol, 700 g. of urea were added and refluxed for 1/2 an hour. After standing overnight, 550 g. of the inclusion compound could be sucked off. Its decomposition with acidified water furnished 104 g. of a fatty acid melting at about 63° C. By adding 300 g. more of urea to the mother liquor only 12.2 g. more of fatty acid could be isolated from the inclusion compound formed. This batch also melted at approximately 63° C.

Conversion into the anilides.—17.5 g. of the crude but thoroughly dried soluble fatty acids were heated together with 7.7 ml of phenyl isocyanate in a round bottom ground joint flask closed with a calcium chloride drying tube with a capillary opening. By melting the fatty acids on water bath the two liquids were made uniform by shaking. On boiling the mixed anhydride of phenyl carminic acid with the fatty acids it solidified. Its decomposition was performed by heating it up to 180-200° C. in a glycerine bath. The effervescence stopped after a few minutes, but the heating was continued for 1 hour.

Then vacuum was used to remove the small excess of phenyl isocyanate and the solidified contents of the flask were pressed on a porous plate. Received: 23.2 g. crude anilides, m.p. 80° C.

Purification by Recrystallisation.—5.52 g. of the crude anilides were dissolved in 100 ml. of ethanol, filtered from impurities, concentrated to approximately 50 ml. and cooled. 3.1 g. were received., m.p. 88-90° C.

Again recrystallised from alcohol. Received 1.55 g., m.p. 90° C. This melting point did not change further even after two more recrystallisations. The analysis furnished: C, 81.76; 81.47; H, 11.30; 11.22; N, 3.64; 3.52; Palmitic anilide $C_{22}H_{37}ON$ (m.p. 90.5° C.) requires: C, 79.70; H, 11.25; O, 4.83; N, 4.23; while Stearic anilide, (m.p. 94° C.) requires: C, 80.22; H, 11.42; O, 3.89; N, 4.45;

The anilide was, therefore, recrystallised twice from alcohol without changing the melting point of 90° C. The values found thereafter were: C, 81.19; H, 12.00; O, 3.79; N, 3.40. Obviously there must still be some impurity in it which, having the same solubility, cannot be removed by recrystallisation. Therefore, further purification was tried by adsorption on alumina.

Purification by adsorption on alumina.—7.48 g. of the crude anilides were dissolved in 70 ml. of alcohol and passed through a column of 20 cm. length containing 200 g. of alumina. (Brockmann). Eight fractions were collected according to Table 4. The first two with alcohol, the others with ethyl acetate as eluent.

TABLE 4.

Frac. No.	Amount	Remarks
1.	150 cc.	4.342 g. Somewhat yellow crystals.
2.	400 cc.	0.493 g. Faintly yellowish crystals.
3.	200 "	0.422 g. Colourless crystals
4.	500 "	0.729 g. " "
5.	1000 "	0.684 g. " "
6.	1000 "	0.168 g. " "
7.	2000 "	0.216 g. " "
8.	2000 "	0.311 g. " "
Total		7.365 g.

Fractions 1 and 2 were mixed and repeatedly recrystallised from alcohol when the melting point kept constant at 90°C. Fractions 3 and 4 were also mixed and melted after one recrystallisation at 89.5 - 90°C. But even three more crystallisations did not raise the melting point above 90°C. Fractions 5-8 were also mixed and recrystallised from alcohol. Here the melting point rose already after the first recrystallisation up to 90-92°C. but kept constant at 94°C. after two more

recrystallisations from alcohol. Stearic anilide melts at 94°C. The combined substances with the melting point 90°C. were again boiled with some charcoal in alcohol. But even then the melting point did not show any rise. This substance, therefore, was considered to be palmitic anilide, with m.p. 90.5°C. The nitrogen analysis furnished: N, 4.16 and 4.17 against N, 4.23 required by palmitic anilide.