STUDIES ON CENTAUREA BEHEN LINN. (COMPOSITAE). PART I

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According to Pharmacographia Indica, Vol. II, p. 303, *Centaurea behen* grows in Iran, Syria and Armenia in two varieties, the white and the red bahman, the roots of which were used as drugs by the ancient Persians. It is mentioned further that the white bahman is free from alkaloids, while the red one contains a bitter tasting alkaloid named bahamine showing an opal blue fluorescence in ether solution from which it crystallises in feathery crystals. Neither the melting point nor analysis or any other data is given.

In Nadkarnis' Indian Materia Medica, *Centaurea behen* is also stated to contain this alkaloid bahamine, but no differentiation is made between the red and the white variety. On the other hand, Nadkarni refers to *Salvia haematodes* W., named 'behen' in Arabic, to contain the same alkaloid bahamine. The roots taken as a powder together with sugar allegedly cure jaundice and calculus affections, and are reported to be aphrodysiac.

Later on, the *Centaurea* species have been investigated by several workers. In 1940 Charaux and Rabate[1] reported that the fresh leaves of *C. scabiosa* contain 2% of a glucoside, the hydrolysis of which furnished glucuronic acid and a flavonol identified as scutellareol. Comparative studies showed the identity of this glucuronoside with scutellaroside. In the same year Dionis and Chermonaz[2] also detected alkaloids and glucosides in *C. diffusa* and *C. inuloides*, while *C. americana* contains, according to Moran, Briex and Couch,[3] 5.8 mg. free hydrogen cyanide per 100 g. of its seeds.

Neutral, acidic and alkaline aqueous extracts of *C. salmantica Linn.* were tested as blood sugar lowering agents. As a result an aqueous glycerol extract has been recommended orally in human diabetes by Aquilar and de Gregoria Rocansolano.[4] A crystalline unsaturated lactone, behenin, m.p. 79-80° C., tetrabromo derivative, m.p. 67° C., with the formulas $C_{24}H_{48}O_3$ and $C_{24}H_{48}Br_4O_3$, respectively, has been isolated from *C. behen Linn.* by Bhargava and Dutt.[5] Lofgren[6] isolated two carotinoid pigments from the stalks and leaves of *C. cyanus*. Bigorra, Lobo, Puig and Yufera[7] observed that aqueous extracts of several *Centaurea* species showed hypoglycemic action when injected intravenously. Numata detected ascorbic acid in several *Compositae* species. In *Centaurea* it varies from 3.2-91.5 mg. %. Finally in 1954 Zolotnitskaya[9] reported that all *Centaurea* species of Armenian flora give a general alkaloid reaction.

Through the courtesy of Hakim Mohammad Said of Hamdard Dawakhana, Karachi, fresh roots of white bahman were made available for the present investigation. It may be mentioned that they were found to contain an alkaloid, in contradiction to the report given in Pharmacographia Indica. The roots were cut into small pieces and extracted exhaustively with petroleum ether of b.p. 60-80° C. A fairly large amount of a slightly brownish, honey-like residue remained after removing the solvent and started depositing crystals on standing.

Notwithstanding these crystals, it was subjected to adsorption analysis on alumina (Brockmann, E. Merck) with petroleum ether, benzene and chloroform subsequently used as eluants. Preliminary tests made it feasible to combine the 95 fractions collected into 7 different groups.

Isolation of Taraxasterol Acetate.—The 4th group consisted of colourless crystals mixed with an oily material and was therefore investigated at first. Recrystallisation nine times from ethyl acetate furnished the pure substance showing the m. p. 249-50° C. and $[\alpha]^{27}_D = +99°$ in 1% cholorform solution. Analysis: C, 81.70; H, 11.15; O, 7.15; m.w. 428 (Rast, camphor). This would agree with a formula $C_{32}H_{52}O_2$, which requires C, 82.05; H, 11.11; O, 6.84. The infrared spectrogram (Fig. 1) showed a strong band between 1700 and 1800 cm.$^{-1}$ suggesting a carbonyl group, and another one at approximately 1250 cm.$^{-1}$ indicating a $-C-O$ bond. Neither aldehyde nor ketone reactions were positive, while at the other side the hydroxamic acid test was clearly positive. From these observations, an ester group was concluded. The substance furthermore must carry at least one double bond because acidified potassium permanganate was decolourised at once in the cold and bromine added in chloroform or carbon tetrachloride solution furnished a bromo derivative (melting at 211°C.) from acetone.

In accordance with the suggestion of the presence of an ester group, hydrolysis with alcoholic alkali led to a colourless substance melting at 220°C. from petroleum ether having still the

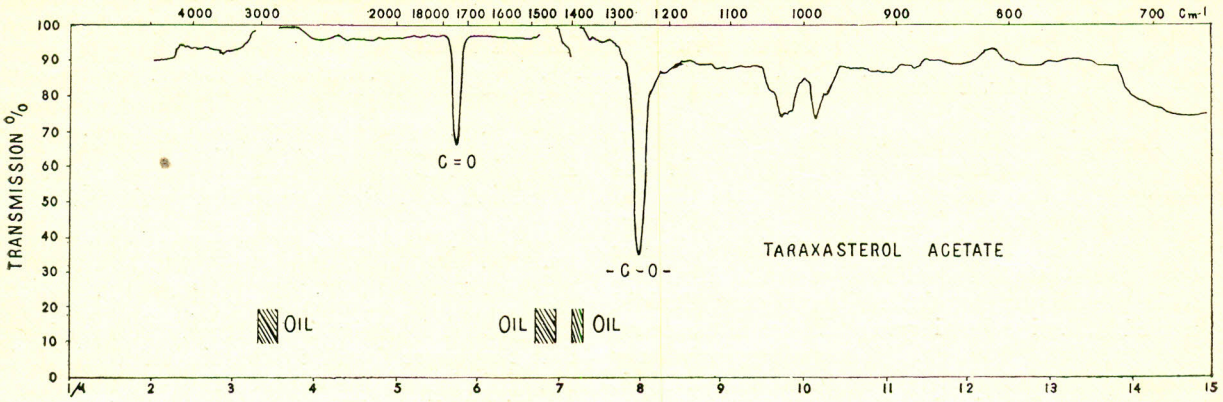


Fig. 1.—Infrared spectrogram of taraxasterol acetate.

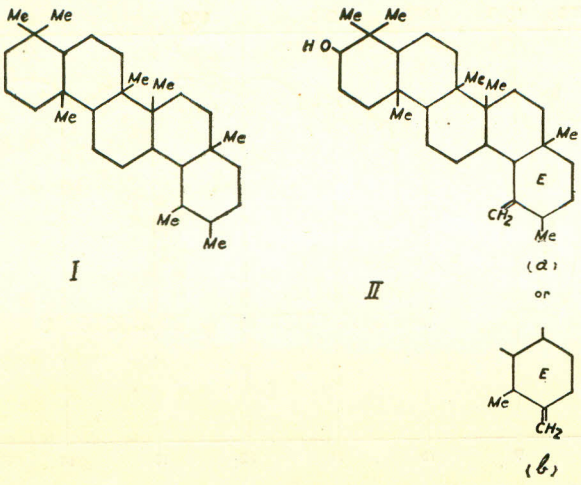
TABLE I

	m.p. °C.	Crystal structure	$[\alpha]_D$ 1% $CHCl_3$	Double bond reactions	Br. deriv. m.p. °C.	Liebermann test
Main subst. group 4	249–50	Colourless hexagon plates	+99°	Positive	211	Negative
Taraxasterol acetate	250–51	,,	+97°	,,	211	,,
Hydrolysate of subst. group 4	220	Colourless needles	+98°	,,	—	Positive
Taraxasterol	221–22	,,	+97°	,,	—	,,

same rotation (namely $[\alpha]^{35}_D = +98°$ in 1% chloroform solution) as the starting ester. The analysis C, 84.67; H, 11.61; O, 3.92 would agree with $C_{30}H_{50}O$, which requires C, 84.5; H, 11.73; O, 3.76. The difference between the two formulas shows a loss of C_2H_2O, which indicates an acetate. This was confirmed by the determination of acetic acid by several methods described in the experimental part. The Liebermann-Burchardt test, negative with the original ester, was now positive, giving a change in colour from red to violet and then (after 1 day) into green. This is indicative of a hydroxy polycyclic compound. Consultation of Elseviers Encyclopaedia of Organic Chemistry leads to taraxasterol and its acetate, respectively. Table 1 shows the conformity of all the data.

Isolation of Taraxasterol.—Group 6 of the eluates was next taken up for purification. The colourless crystals of this group melted between 145-165 °C., and could not be purified further by adsorption analysis on alumina. Recrystallisation was the only possibility, but, even after 20 recrystallisations from petroleum ether (alcohol and ethyl acetate, though furnishing much better shaped crystals, were less effective), the melting point, which had reached 217-18 °C., was not yet constant. The rotation however ramained constant at $[\alpha]^{30}_D = +97°$ in 1% chloroform solution, which figure is identical with that of taraxasterol. An analysis of the material melting at 205-208 °C. furnished C, 83.85; H, 11.61; O, 4.65 while taraxasterol $C_{30}H_{50}O$ requires: C, 84.5; H, 11.73; O, 3.67. The difference seems to indicate that the substance or substances that are connected with each other so obstinately in the product are not isomers. Taking into account the still rising melting point and the constancy of the rotation at the correct value, together with the fact that only taraxasterol besides its esters and ψ-taraxasterol (from group 1) could be isolated so far, it is reasonable to conclude that the main substance of group 6 is taraxasterol.

Jeger,[10] Lardelli,[11] and Dietrich[12] have shown that taraxasterol, ψ -taraxasterol, arnidiol and faradiol have the same skeleton as heterobetulin, which is obtained from betulin by isomerisation processes. All these compounds derive from the saturated hydrocarbon, heterolupane I.



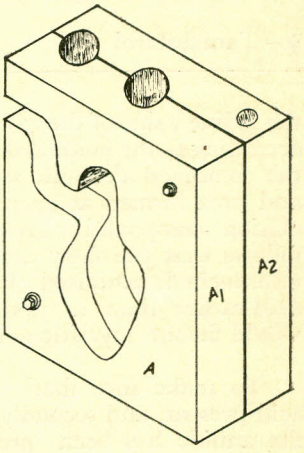
Taraxasterol II has been tentatively described to be 2,hydroxy Δ–19: 29 (a) or Δ–20:30 heterolupane (b). Taraxasterol occurs in the white rays of marguerite flowers also belonging to the same family *Compositae*. It was further isolated from the dried latex of *Lactuca virosa* and from *flos chamomilla romanae*, the flowers of *Anthemis bobilis*. In the latex of *Calotropis procera* (*Asclepiadaceae*), it occurs as acetate, isovalerate, pyroterebate, and probably other esters. It was futher isolated from the latex and the stem bark of *Centaurea gigantea*.

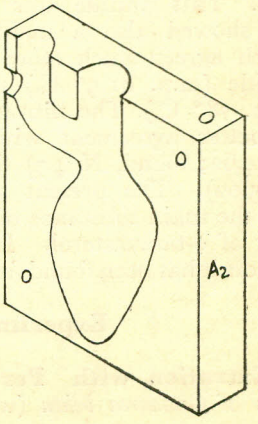
Isolation of the Myristic Ester of ψ-Taraxasterol.—The first group was received from the column as a water-clear colourless oil of high viscosity. It could be distilled in a copper block * at about 325 °C. 0.01 mm. Hg without colouration. Even the small amount of substance remaining in the distillation flask remained as colourless as before.

After standing for several months in air, the material became slightly yellow. The raw material was found to be neutral and free from fatty acids. The distillate on analysis gave the following values: C, 83.65; H, 11.89; O, 4.69. The infrared spectrogram (Fig. 3) showed a strong band between 1700 cm.$^{-1}$ and 1800 cm.$^{-1}$ suggestive of a carbonyl group, and another one at 1250 cm.$^{-1}$ indicative of a –C–O–bond. A small test confirmed that both groups are combined in an ester grouping. On saponification of the raw material with alcoholic alkali, a colourless crystalline substance could be obtained as the alcoholic part of the ester besides fatty acids. It was not possible to purify it by adsorption analysis, and it had to be recrystallised 15 times until the melting point was constant at 215-216 °C. The rotation also was constant at $[\alpha]_D^{38} = +51°$ in 1% chloroform solution. It decolourises permanganate as well as bromine solution, and shows a series of transient colours with Liebermann-Burchard solution. Its analysis furnished: C, 84.6; H, 11.6; O, 3.8, in agreement with $C_{30}H_{50}O$, which requires: C, 84.5; H, 11.73; O, 3.76. On consulting Elseviers Encyclopaedia of Organic Chemistry the compound was found to be ψ–taraxasterol, which could be confirmed by preparing the acetate. Table 2 shows the conformity of the data.

ψ-Taraxasterol is known to occur always together with taraxasterol, from which it differs only in having the double bond within ring E, either between C 19:20 or 20:12. By refluxing taraxasterol with 10% alcoholic sulphuric acid in benzene for 5 hrs. it can be isomerised to ψ-taraxasterol.

Identification of the Fatty Acid with Myristic Acid $C_{14}H_{28}O_2$.—The melting point of the recrystallised fatty acid was found to be 47-49 °C. (from petroleum ether). Therefore both lauric acid (m.p. 43.6 °C.) and myristic acid (m.p. 54 °C.) had to be taken into consideration because





* For the distillation of very small amounts of substances, the apparatus shown in the figure has been developed and found to be very useful. The copper or brass block A is cut in two halves, A_1 and A_2, which can be put together by bolts. Inside the block, a recess is shaped to receive a small Claisen distillation flask of 2—10 ml. capacity with the capillary C and a short thermometer. An aperture of the same shape as the distillation flask in the front half of the block allows observation of the distillation. The copper block is heated by an open flame and provides uniform and constant heat up to that point where the vapours leave the distillation flask so that no overheating occurs.

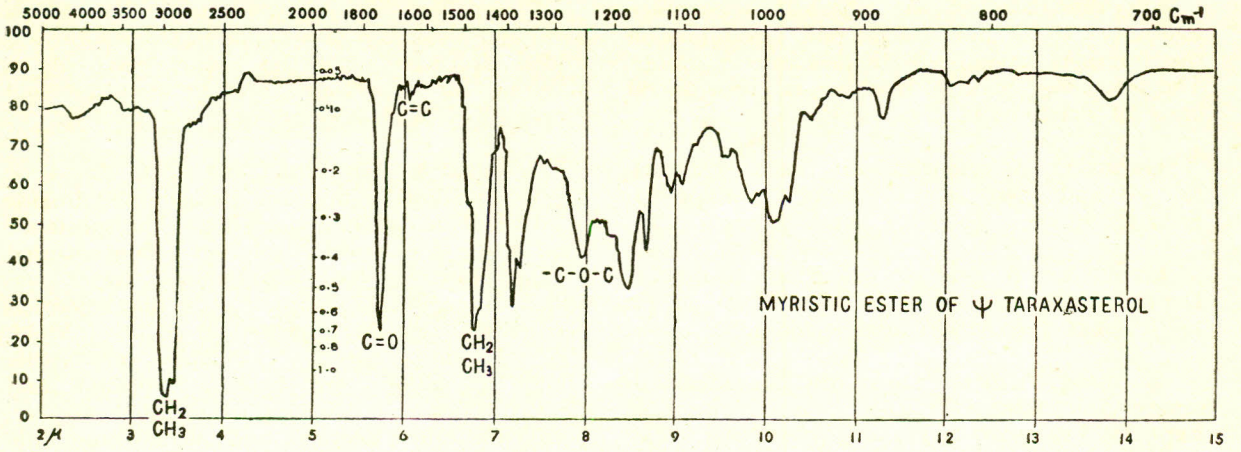


Fig. 2.—Infrared spectrogram of myristric ester of ψ-traxasterol.

TABLE 2

	m.p. °C.	Crystals	$[\alpha]_D$ 1% $CHCl_3$	Liebermann test	m.p. of acetate °C.
Hydrolysate of group 1	215–16	Needles	+51°	Positive	234–36
ψ – Taraxasterol	216–17	,,	+50°	,,	238

the iodine value of the raw acid was found to be 35 according to the method of Hanus, and furthermore the combined alcoholic solutions of the fatty acid and urea formed at once a nicely crystallised inclusion compound. From both observations it follows that the fatty acid must have a straight, unbranched, saturated chain. Assuming a single acid rather than a mixture, the melting point would favour myristic acid.

To make sure firstly that there is only one acid present, and secondly that it is myristic acid, the anilide has been prepared via the mixed anhydride of the fatty acid with phenylcarbaminic acid. This anilide was not only homogeneous, but showed also a melting point of 81-82° C. which agrees much more with that of myristic anilide (m.p. 81.5° C.) than with lauric anilide (m.p. 78° C.). The nitrogen values found are also in better agreement with the myristic anilide (N, 4.62; found, N, 4.7) than with lauric anilide (N, 5.02). The present authors therefore think that the main substance of group 1 is the myristic ester of ψ-taraxasterol. In the knowledge of the authors it has been found for the first time in nature.

Experimental

Extration with Pertoleum Ether.—Fresh roots of *Centaurea behen* (white variety) were cut into small pieces of 1/4″-1/2″ length and extracted three times with petroleum ether; b.p. 60-80° C.

Experiment I: 1.6 kg. roots, on extraction with 6 litres of petroleum ether, yielded 15.73 g. of solvent free extract (0.98% of the fresh roots or 1.1% of the dried roots, which contained 11% moisture).

Experiment II: 7 kg. fresh roots, on extraction with 20 litres of petroleum ether, yielded 56.5 g. extract (0.81% of the fresh and 0.93% of the dried roots, respectively, which contained 13% moisture).

Adsorption Analysis on Alumina (Brockmann, E. Merck).—*Experiment I:* 15.5 g. of the brownish, sticky mass was dissolved in a minimum quantity of petroleum ether and placed on top of a column of 50 cm. length, 4 cm. width, filled with 480 g. of alumina. As eluants, petroleum ether, benzene and chloroform were used successively. Ninety five fractions of 50 ml. each were collected. Total eluates received, 12.826 g.; remained in the column, 2.674 g.; total, 15.5 g.

Experiment II: 55 g. extract. Column had 4 cm. width, 90 cm. length and was filled with 800 g. of alumina. Fifty eight fractions of 50 ml. each were collected. Total eluates received, 47.637 g.; remained in the column, 7.363 g.; total 55.0 g.

On the basis of the physical appearance, solubility tests and microscopic examination, the fractions have been divided into 7 groups. The percentage yield of fractions is given on the basis of dried roots.

GROUP 1: A colourless oily mass. Expt. I, Fr. 5-8 and 17-20, 4.712 g.(0.33%); Expt. II, Fr. 1-13, 28.112 g. (0.46%); total, 32.824 g.

GROUP 2: A gel-like colourless substance. Expt. I, Fr. 9-16, 2.567 g. (0.18%); Expt. II, Fr. 14-16, 1.231 g. (0.02%); total, 3.799 g.

GROUP 3: A mixture of an oily substance with some crystals. Expt. I, Fr. 21-27, 0.617 g. (0.043%); Expt. II, Fr. 17-20, 1.173 g. (0.019%); total, 1.790 g.

GROUP 4: Mainly a crtystalline colourless substance. Expt. I, Fr. 28-80, 2.201 g. (0.15%); Expt. II, Fr. 21-50, 5.574 g. (0.092%); total, 7.775 g.

GROUP 5: An oily brownish substance. Expt.I, Fr. 81-92, 1.022 g. (0.07%); Expt. II, Fr. 51-53, 2.398 g. (0.039%); total, 3.420 g.

GROUP 6: Colourless needle shaped crystals. Expt. I, Fr. 93-94, 1.021 g. (0.017%); Expt. II, Fr. 54-56, 3.666 g. (0.06%); total, 4.687 g.

GROUP 7: A semisolid brownish mass with some crystals. Expt. I, Fr. 95, 0.449 g. (0.03%); Expt. II, Fr. 57-58, 2.156 g. (0. 03%); total, 2.605.

Isolation of Taraxasterol Acetate from Group 4.—The melting points of the several fractions from the first experiment are given in Table 3.

TABLE 3

Fr.	m.p.°C.	Fr.	m.p.°C.
28	150–195	58	214–219
33	190–216	63	215–220
38	211–216	68	217–220
43	212–218	80	219–221
53	212–218		

Fractions 58-80 were combined and recrystallised from ethyl acetate. After nine recrystallisations the m.p. remained constant at 249-50°C. The rotation in 1% chloroform solution was also constant at $[\alpha]^{27}_D = +98$°C. Found: C, 81.70; H, 11.15; O, 7.15. $C_{32}H_{52}O_2$ requires: C, 82.05; H, 11.11; O, 6.84. The infrared spectrogram is shown in Fig. 1.

Bromination.—100 mg. were dissolved in chloroform and a solution of bromine in chloroform was added dropwise until the colour persisted on standing for one hour. Solvent removed and recrystallised from acetone, m.p. 211°C. dec.

Hydrolysis to Taraxasterol and Acetic Acid.—200 mg. were refluxed with 40 mg. potassium hydroxide in 20 ml. of alcohol for about 9 hrs. The substance went slowly in a colourless solution. Concentrated to approximately half the volume, water was added to precipitate completely the fine colourless needles which already appeared on cooling. Yield, 180 mg., m.p. 210-213°C. They were easily soluble in chloroform, benzene, dioxane and tetrahydrofurane, sparingly so in ethyl acetate, petroleum ether, acetone and alcohol, insoluble in water. Recrystallised from petroleum ether m.p. 220°C. $[\alpha]^{35}_D = +98$ in 1% chloroform solution. Found: C, 84.67; H, 11.61; O, 3.92. Taraxasterol $C_{30}H_{50}O$ requires C, 84.5; H, 11.61; O, 3.92.

The alkaline mother liquor was brought to dryness (61 mg.) and submitted to spot tests for carboxylic acids (hydroxamic acid test), test for acetate, and finally for acetic acid itself according to Feigl. These tests were positive for acetic acid. Finally the rest of the salt was acidified and heated. The acetic acid could also be confirmed by the smell.

Isolation of Taraxasterol.—The colourless needle-shaped crystals of group 6 melted at 145-165°C. They were easily soluble in chloroform, benzene, dioxane and tetrahydrofurane; sparingly so in ethyl alcohol, methyl ethyl ketone, acetone, petroleum ether, ethyl acetate, cyclohexane and glacial acetic acid, insoluble in water. The purification was done by recrystallisation from several different solvents, among which petroleum ether was found to be the most effective, although the crystals from ethyl acetate or alcohol were much better shaped. Adsorption analysis on alumina failed to separate the mixture. Table 4 shows the slow rise in melting point for the successive recrystallisations.

The rotation obtained for No. 11 was $[\alpha]^{30}_D = +97°$ in 1% chloroform solution. With the Liebermann-Burchardt solution, the product gave a pink colour, which deepened slowly to purple and changed through violet and pale blue to light green during the next few hours. Potassium permanganate and bromine solutions were decolourised in the cold. Though all these data agree with taraxasterol, an analysis done with No. 11 (Table 5) showed a remarkable difference especially in the oxygen value and somewhat

TABLE 4

Recryst. No.	m.p.°C	Solvent
1	165– 77	Petroleum ether
2	178– 84	Ethyl acetate
3	184– 92	Petroleum ether
4	189– 97	,,
5	192– 98	,,
6	193– 98	Alcohol
7	195–200	Petroleum ether
8	197–202	,,
9	199–203	,,
10	203–207	,,
11	205–209	,,
after 19	215–217	,,
20	217–218	,,
21	217–218	,,

less in the carbon content, thus making it doubtful that the substance connected so obstinately with taraxasterol could be an isomer.

Found: C, 83.85; H, 11.61; O, 4.65. Taraxasterol requires: C, 84.5; H, 11.73; O, 3.76.

Isolation of the Fatty Acid Ester (Myristic) of ψ-Taraxasterol.—The clear, colourless oily substance of group 1 was distilled under high vacuum. To prevent overheating, the copper block already described has been developed. The oil distilled at 325°C., 0.01 mm, the high vacuum being produced by using an oil pump as pre-vacuum to a mercury pump. It is remarkable that no colouration took place even in the small amount of residue in the distillation flask.

The distillate gave the following values on analysis: C, 83.65; H, 11.89; O, 4.69. ψ-Taraxasterol myristic ester $C_{44}H_{76}O_2$ requires: C, 82.95; H, 12.03; O, 5.02; while the corresponding lauric ester $C_{42}H_{72}O_2$ requires: C, 82.83; H, 11.92; O, 5.25.

Saponification of the Myristic Ester of ψ-Taraxasterol.—16 g. of the raw oily substance of group 1 was refluxed with 20 g. of potassium hydroxide in 110 ml. alcohol. The oil went slowly into solution, which became dark red. After 13 hrs. boiling, it was cooled down and a few ml. of water were added in order to precipitate completly the fine colourless needles which appeared on cooling. They were filtered by suction, washed with a few ml. of an alcohol-water mixture (1:1) and dried on a porous plate. Yield, 5.15 g., mp. 155-162°C.

The red alkaline mother liquor was concentrated to about one third of its volume, so that the whole of the alcohol was driven off. The remaining solution was acidified with hydrochloric acid and the material coming out was taken up exhaustively with petroleum ether. After washing and drying, the petroleum ether was removed. Residue, 10.02 g.

A reddish brown substance still floating in the solution was taken up with amyl alcohol and found to give 0.8 g. of a reddish brown solid material.

The above mentioned residue of 10.02 g. was found to contain alkali insoluble material. It was refluxed for 3 hrs. with 10 g. potassium hydroxide in 80 ml. of alcohol, the alcohol was removed and the residue exhaustively extracted with petroleum ether. On removing the petroleum ether from the washed and dried solution, 4.8 g. of unsaponifiable substance of a glassy, brittle consistency was obtained. The petroleum ether insoluble portion, the soap, was dissolved in water and acidified. The fatty acids obtained were taken up in petroleum ether and yielded 5.1 g.

Purification and Identification of ψ-Taraxasterol.—The above received 5.15 g. of a sterol was dissolved in petroleum ether and passed through a column of alumina. But the outcoming eluates with different solvents revealed that no clear separation could be achieved. The regained substance therefore was subjected to recrystallisation from alcohol and petroleum ether successively. Table 5 shows the results.

The rotation of the last two fractions was also constant and showed $[\alpha]^{38}_{D} = +51°$ in 1% chloroform solution. The analysis agreed with the formula $C_{30}H_{50}O$ which requires C, 84.5; H, 11.73; O, 3.76. Found: C, 84.6; H, 11.6; O, 3.8;

The needle shaped crystals also gave a series of transient colours with the mixture of concentrated sulphuric acid and acetic anhydride (Liebermann-Burchardt test) and decolourised bromine and permanganate solutions.

ψ-Taraxasterol Acetate.—100 mg. of the substance, m.p. 215–216°C., was refluxed with 2 ml. of acetic anhydride and two drops of pyridine for about 3 hrs. Water was added to the still hot solution and cooled. The outcoming crystals were filtered by suction and dried on a

TABLE 5

Recrystl. No.	m.p. °C.	Solvent
1	165– 72	Alcohol
2	180– 87	,,
3	186– 93	,,
4	188– 95	,,
5	193– 97	Petroleum ether
6	197–202	,,
7	200–204	,,
8	202–205	,,
9	204–207	,,
10	206–209	,,
11	209–212	,,
12	211–214	,,
13	213–215	,,
14	215–216	,,
15	215–216	,,

porous plate, m.p. 234-236 °C. (three times from ethyl acetate). The plates are easily soluble in chloroform and benzene ; moderately so in ethyl acetate, petroleum ether and alcohol ; insoluble in water. ψ-Taraxasterol acetate melts at 238°C.

Identification of the Fatty Acid with Myristic Acid.—On recrystallisation of a small amount of the above received 5.1 g. of fatty acids from petroleum ether, the melting point was found to be fairly sharp at 47-49 °C.

The iodine value of the raw material to be determined according to the method of Hanus was found to be 35. The acid therefore must be a saturated one.

The combined solutions of the fatty acid and urea in alcohol form at once a nicely crystallising inclusion compound. It follows that the chain of the fatty acid must be a straight one. The melting point of lauric acid is 43.6 °C. while myristic acid melts at 54 °C.

Preparation of the Anilide.—2.4 g. of the dry fatty acid (raw material) was melted, 1.5 ml. phenyl isocyanate added and mixed by shaking in a round bottom flask closed with a calcium chloride tube by ground glass joint. Notwithstanding the outcoming crystals of the mixed anhydride of fatty acid with phenyl carbaminic acid, the mixture was heated in a glycerine bath until the effervescence stopped. The excess of phenyl isocyanate was then removed by using vacuum at 110-120°C. for approximately 1 hr. Afterwards the solidified anilide was pressed on a porous plate. Yield, 2.8 g. On recrystallisation from alcohol-water it turned out that all fractions melted sharply at 81-82°C. It follows that there is only one fatty acid present. The melting points of the anilides of lauric and myristic are 78 °C. and 81.5 °C. respectively. Finally the nitrogen value was determined and found to be 4.7. The nitrogen value of lauric anilide is 5.02 as against 4.62 for myristic anilide.

Taking into consideration that nitrogen values tend always to be on the high side, the value would rather agree with myristic anilide than with lauric anilide. The same conclusion can be drawn from the melting points of free fatty acid as well as of their anilides. The present authors therefore think that the main substance of group 1 is the myristic ester of ψ-taraxasterol.

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