

STUDIES ON LAVANDULA STOECHAS LINN. (LABIATAE). PART I

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A hydrocarbon most probably *n*-nonacosane, *d*-Camphor and three sterols so far unknown were isolated from the petroleum ether extract of *Lavandula stoechas* Linn. The unknown sterols were named as Lavandula Sterols A, B & C, having two, one and three oxygens in their respective formulae. Specially the one with three oxygens attracts interest as starting material for cortisone synthesis because when the plant is harvested during special time of the year it contains as much as 2.8% of this sterol C.

Introduction

As mentioned in Nadkarni's Indian Materia Medica, *Lavandula stoechas*, Linn. (Labiatae) is used under the name of Ustukhudus in Unani drugs to cure chest affections, and to relieve biliousness. The plant is also known as Dharu and Alphajana Dharu in Hindi. In antiquity Dioscorides states that this plant is called stoechas, because of growing on the Stoechades, a group of islands on the south coast of Gaul near Massilia, now called Isles d'Hyeres. In Western India, the drug is best known (though incorrectly) under the Portuguese name of Alphazema which is corrupted by natives into Alphajana.¹ In English it is known as French 'avender.

Dymock, Warden and Hooper have mentioned in Pharmacographia Indica that the plant is much used by the Muslim physicians who consider it to be cephalic, deobstruent, and carminative and prescribe it in chest affections. They also think that it assists in expelling bilious and phlegmatic humors. The author of Makhzen-el-Adwiya devotes a whole folio page to a description of its properties, and especially enlarges upon its cephalic virtues. He concludes by saying, "In short Ustukhudus is the broom of the brain; it sweeps away all phlegmatic impurities and removes obstructions, strengthening its power, expelling vain crudities and rarifying the intellect." *Lavandula stoechas* is known in Spain as Rosemero Santo, (sacred rose-mary). Its essential oil is obtained there for household use by suspending the fresh flowering stalks, flowers downwards, in closed bottles, and exposing them for some time in sun rays. A mixture of water and essential oil collects at the bottom, which is used as a haemostatic and for cleaning wounds.

Investigation

The preliminary investigation of the plant (stems only) was done with only 300 g. which furnished approximately 3% petroleum ether extract and 5% alcoholic extract. Specially from the petroleum

ether extract, needles and a white amorphous substance could be obtained while besides an essential oil there was also present the characteristic smell of camphor. Therefore it was decided to start a fuller investigation of this plant.

The dried whole plant without roots (3.6 kg.) was exhaustively extracted with petroleum ether. Eleven extractions using in all 86.5 litres of petroleum ether (b.p. 60-70° C.) in 96 days had been necessary. On concentrating the various fractions, an amorphous greenish substance settled down which was separated from each fraction by suction on filter paper. A total of 103.03 g. of this amorphous substance equivalent to 2.8% of the plant material could be obtained while the amount of petroleum ether extract totalled up to 306.061 g., equivalent to 8.5% by weight of the plant material.

When different extracts, after removing the sparingly soluble greenish amorphous substance, were concentrated, crystals started depositing. Notwithstanding these crystals, it was subjected to adsorption analysis on alumina (Brockmann, E. Merck). The total extracts (the soluble part of petroleum ether) (83.791 g.) were dissolved in 500 ml. of petroleum ether and poured into a column of 12" length and 3-1/2" width packed with 1500 g. of Brockmann alumina (E. Merck). The ratio of the substance to adsorbant was therefore 5.5%, while the classical adsorption analysis prescribes only 2-4%, but it has been found that for larger quantities, fairly good separation could be achieved with these higher percentages. Petroleum ether, benzene, ethyl acetate, and chloroform had been used successively as eluents. One hundred and thirty fractions were collected, and in all 43.7948 g. of the substance was recovered, which means that nearly half of the starting material, namely 39.9962 g., must be still in the column. This is still under investigation. Preliminary tests made it feasible to combine the 130 fractions into four different groups, and each group was investigated separately.

Isolation of Lavandula Hydrocarbon.—Out of 5.4017 g. of the substance from Group I, a solid

substance melting at 58-61° C. was obtained by high vacuum distillation at 0.02 mm. pressure and at 208-240° C. On recrystallization this solid substance from acetone, a brittle white substance, having the constant m.p. 61-63°C., was obtained, analysis of which furnished the values C, 85.54; H, 14.59. This would agree with a straight chain hydrocarbon of the formulae: $C_{27}H_{56}$ requiring C, 85.17; H, 14.83; m. p. 60° C.; or $C_{28}H_{58}$ requiring C, 85.19; H, 14.81; m.p. 61.6° C.; or $C_{29}H_{60}$ requiring C, 85.20; H, 14.80; m.p. 64° C.; because the original substance furnishes very nicely shaped crystals of urea inclusion compound, out of which on decomposition with water, the starting material is received back as a waxy substance floating on the surface, while urea goes in solution. The substance does not show any rotation in 1% chloroform solution. It seems to be saturated because a chloroform solution of bromine left the substance unchanged even on standing for several hours and also potassium permanganate solution was not decolorized in neutral, acidified or alkaline medium. According to the m.p. of 61-63°C., the lavandula hydrocarbon is most probably *n*-nonacosane.

Isolation of d-Camphor.—While collecting the fractions, it was noticed that the first fraction of the group II, smelled fairly strongly of camphor. Therefore the solvent from the fractions composing the group II, was removed first by distillation under reduced pressure, and afterwards by keeping the flasks in a dessicator evacuated by an oil pump, so that the small amount of remaining solvent, i.e. the petroleum ether, was removed completely. It was then thought feasible to sublime the whole camphor present in the group on a cooling finger, by introducing it by means of an adaptor. By slightly warming the bottom of the conical flask, the camphor was soon sublimed on the cooling finger in absolute purity. It was characterized by taking the melting point, which was found to be 172-173°C. (in a sealed tube) whereas the literature mentions 176°C. and 179°C. for *d*-camphor, and the rotation in 1% alcoholic solution, which was found to be +43°, while the literature mentions +44°. Besides its characteristic smell, a small crystal obtained by sublimation from group II when put in a drop of water on a slide, and observed under microscope, was found to be rotating and vibrating vigorously, which is a property of camphor.

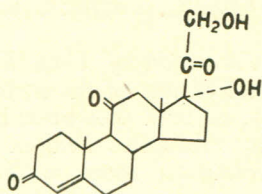
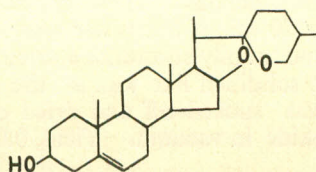
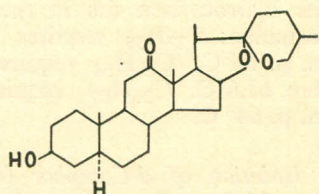
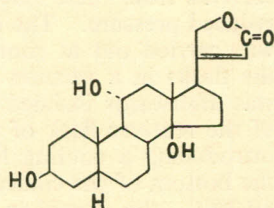
Isolation of Lavandula Sterols A and B.—Group III of fractions consisted of a yellowish oil, which crystallized on adding petroleum ether and keeping it standing. As described in the experimental part, two sterols could be isolated. The lavandula sterol A, having m.p. 204-205° C., $[\alpha]_D^{29} = +39.6^\circ$ in 0.5% chloroform solution, furnished C, 80.39;

H, 10.08; O, 9.37, fitting in the narrow range of $C_{22-23}H_{32-34}O_2$ only; and the lavandula sterol B, having the m. p. 135-136°C, $[\alpha]_D^{24} = -23.5^\circ$ in 1% chloroform solution, C, 83.2; H, 11.86; O, 4.94% (by difference), the molecular formula fits into the range of $C_{22}H_{36-38}O$. Both the sterols gave the positive Liebermann and digitonin tests, but they could not be identified with any known sterols. Being present in very small quantities, no further characterisation work was carried out at this stage.

Isolation of Lavandula Sterol C.—The greenish amorphous substance which settled down on concentration of the petroleum ether extract was, after purification and crystallization, proved to be a sterol, which has been named lavandula sterol C, because it could not be so far identified with any known one. In crude form, it was highly contaminated with chlorophyll and certain other impurities, wherefrom it was difficult to separate. The best method of getting the sterol in the completely white but still amorphous state was soxhlet extraction with chloroform, in which it is sparingly soluble in the cold. Its crystallisation was achieved from ethanol, when the m.p. was constant at 268-270°C., $[\alpha]_D^{27} = +67.2^\circ$ in 1% tetrahydrofuran solution. Analysis report furnished the values, C, 77.42; H, 10.58; O, 12.46, which fits into the molecular formulae, $C_{26}H_{40-44}O_3$.

By boiling with acetic anhydride, it yielded an acetyl derivative, m.p. 278-280° C., $[\alpha]_D^{26} = +65^\circ$, the acetyl value of which was found to be 8.96, accounting for only one acetyl group. The two other oxygen atoms therefore seem to belong to other groupings. Unfortunately, the infra-red spectrogram could not be made available, therefore, the easy method of finding out if the other two oxygens are present as a lactone, ester or keto groups was not applicable. On heating with alcoholic potash, however, the substance is transferred into a difficultly soluble potassium salt of an acid formed by splitting a probable lactone ring in the molecule. The other two oxygens are therefore combined in that lactone group. This fact makes the substance considerably interesting because it might be a good starting material for the cortisone synthesis.

These hormones of the adrenal glands have achieved immense importance due to their striking efficacy in the amelioration of rheumatoid arthritis, allergy and lymphatic leukemia, and in promoting the healing of burns. The technological production of these hormones, especially of cortisone, has become an important industrial problem. The twenty steps previously encountered in the transformation of bile acids into cortisone I, could be considerably shortened by starting

Cortisone
IDiosgenin
IIHecogenin
IIISarmantogenin
IV

from more suitable compounds, e.g. diosgenin II from Mexican jam, and hecogenin III, which has been found in abundance in the juice of Agave groups of plants to which also sisal belongs. According to New Zealand Science Report of January 1959, this source is already used successfully in the Commonwealth. Finally, sarmantogenin IV, which is available from *Strophanthus sarmantensis* may be mentioned. As already pointed out lavandula sterol C is not identical with any of them. In its molecular formula it comes nearest to diosgenin, but in its behaviour towards alcoholic potash, it seems to have a lactone grouping which might be similar to that present in sarmantogenin. Its usefulness in the cortisone synthesis however will depend upon further studies on its constitution which are underway.

Experimental

Petroleum Ether Extraction.—The whole plant without roots (3.6 kg.), completely dried and

crushed to small parts, was extracted exhaustively (11 times) with petroleum ether (b.p. 60°C.).

When the different extracts were concentrated to about 500 to 700 ml. under reduced pressure at 35-40°C. some of the substance settled down which was sucked off. Later experiments showed that it was a sterol which is named as lavandula sterol C. The filtrates of the extracts were individually concentrated, finally dried over phosphorus pentoxide and weighed. The weight of the sterol C was added to the filtrates to get the total weight of the individual extracts.

Different extracts were treated according to the above scheme. The results obtained are given in Table I.

Absorption Analysis on Alumina.—Two experiments were carried out separately.

EXPERIMENT I : 30.8 g. of the first extract (including the lavandula sterol C) was taken up in 120 ml. of petroleum ether (some of the substance remained insoluble, i.e., the lavandula sterol C) and placed on top of a column of 3.5 cm. width and 40 cm. length packed with 350 g. of Brockmann's alumina (E. Merck). Petroleum ether, benzene, ethyl acetate and chloroform were used as eluents successively. Ninety-eight fractions were collected. Total eluents received, 15.6868 g.; remained in column, 15.132 g.; total, 30.8 g.

EXPERIMENT II : 83.701 g. of the combined 2nd to 11th extracts (without the already separated lavandula sterol C) was dissolved in 500 ml. of petroleum ether, and poured into a column of 14"

TABLE I

Extract No.	Amount of solvent	Duration of extraction	Sparingly soluble sterol C	Total weight of extract
1	14000 ml.	75 hrs.	33.83 g.	153.07 g.
2	9500 "	60 days.	15.56 g.	61.49 g.
3	8500 "	70 hrs.	18.41 g.	35.72 g.
4	8500 "	72 "	5.32 g.	14.73 g.
5	7500 "	71 "	3.00 g.	8.34 g.
6	8000 "	70 "	10.41 g.	12.54 g.
7	8000 "	96 "	7.33 g.	8.50 g.
8	8000 "	87 "	3.40 g.	4.35 g.
9	8000 "	106 "	2.72 g.	3.58 g.
10	8000 "	114 "	1.97 g.	2.508 g.
11	8500 "	123 "	1.08 g.	1.233 g.
Total	86.5 l.	96 days	103.03 g.	306.061 g. =8.5 %.

length and 4" width, packed with 1500 g. of Brockmann's alumina (E. Merck). Petroleum ether, benzene, ethyl acetate and chloroform were used as eluents successively. One hundred and thirty fractions were collected. Total eluents received, 43.7948 g.; remained in column, 39.9962 g.; total, 83.791 g.

On the basis of physical appearance, solubility tests and microscopic examinations, the fractions have been combined into four groups as shown in Table 2.

TABLE 2

Fractions	Weight	Group	Remark
Exp. I, 1-4 Exp. II, 1-6	2.6479 g. 5.4017 g.]	I	White waxy material
Exp. I, 5-25 Exp. II, 7-14	3.00225 g. 8.0131 g.]	II	Yellowish oil, containing camphor
Exp. I, 26-58 Exp. II, 15-49	2.34025 g. 8.4699 g.]	III	Yellow oil, crystallises on keeping longer and adding pet. ether
Exp. I, 59-98 Exp. II, 50-130	7.5964 g. 21.9101 g.]	IV	Greenish yellow oil

Isolation of Hydrocarbon (Group I).—Fractions 1 to 6 from Exp. II were combined by dissolving in chloroform, and then transferred into a micro-distillation Claisen flask. The solvent was removed completely, and the white waxy substance thus obtained was distilled under high vacuum according to the following scheme:

Starting Temp. material °C.	Pressure mm.	Fractions	Amount g.	Remark
5.4017	120-140	0.05	I	0.258 Oily
g. 168-184	0.03	0.03	II	0.187 "
208-240	0.02	0.02	III	2.731 Solid

The fraction III was dissolved in about 100 ml. of acetone by warming on a water-bath. On cooling a brittle white substance settled down. It was sucked off on a sintered porcelain crucible and dried over phosphorus pentoxide in vacuum. Yield, 1.818 g.; m.p., 58-61°C. After the third re-

crystallization from acetone the m. p. was constant at 61-63°C. Found: C, 85.54; H, 14.59.

Formation of Urea Inclusion Compound.—One hundred mg. of the hydrocarbon mixed with 400 mg. of urea was heated with 10 ml. of butanol. The substances went in solution completely. On cooling to room temperature nice rod shaped crystals came out, which were sucked off. Yield, 0.194 g.; m.p., 132-134°C. The crystals were homogenous. These crystals were then dissolved in 50 ml. of hot water when urea went in solution and an oily substance started floating. On cooling it solidified like wax as the original substance. It was sucked off and dried over phosphorus pentoxide in vacuum. Yield, 0.087 g.; m.p., 59-62°C.

According to the value obtained, the range of the hydrocarbon fits in the following molecular formulae: $C_{27}H_{56}$ requires C, 85.17; H, 14.83; m. p. 60°C. $C_{28}H_{58}$ requires C, 85.19; H, 14.81; m.p. 61.6°C. $C_{29}H_{60}$ requires C, 85.20; H, 14.80 m. p. 64°C.

Isolation of d-Camphor (Group II).—Fractions 7 to 14 from Exp. II smelled like camphor. The solvents from these fractions were removed under reduced pressure. The final removal of the solvent was carried out at room temperature by keeping the flasks in a vacuum dessicator. The camphor was afterwards isolated by direct sublimation out of the receiver flask of the combined fractions by introducing a cooling finger. By slight warming the bottom of the conical flask on a water bath at 60-70°C. the camphor soon condensed on the cooler in absolute purity. Yield, 1.12 g.=0.031% of the plant material.

The sublimed substance exhibited the following properties characteristic of camphor:

(1) The substance has the characteristic smell of camphor. (2) It melts at 172-173°C. whereas *d*-camphor melts at 176°C. (179°C). The melting point was taken in a sealed tube. (3) Mixed m.p. showed no depression. (4) Optical rotation was found to be $[\alpha]_{21} = +43^\circ$ in 1% ethanolic solution. The optical rotation of *d*-camphor as mentioned in the literature is $+44^\circ$. (5) A crystal from the fraction when put in a drop of water on a slide and observed under microscope seemed to be rotating and vibrating vigorously which is a property of camphor.

Isolation of Lavandula Sterol A.—The fractions of the group III of Exp. I were combined together by dissolving in ethyl acetate. The solvent was completely removed, and the yellow oil thus

obtained was dissolved in petroleum ether by heating on a water-bath. It was then concentrated to about 5-7 ml. and kept standing at room temperature for crystallisation. After keeping it overnight, needles started coming out. The mother liquor was decanted and the crystals attached to the walls of the flask were washed twice with a little amount of petroleum ether. The clear crystals after redissolving in petroleum ether by heating on a water-bath were kept again for crystallization. Lengthy rods along with some amorphous substance came out on standing over two nights. The mother liquor was again decanted off from the rods and the amorphous substance, and after concentration left for crystallisation. Some more rods came out. The rods (lavandula sterol A) were separated from the amorphous substance mechanically (yield, 0.0236 g.) m.p.=204-205°C. (on Koflers' block).

The amorphous substance sublimed into well-defined plates, m.p.=114-115°C. on Koflers' block. Further characterisation could not be carried out due to non-availability of more of the amorphous substance.

Analysis report of lavandula sterol A shows C, 80.39; H, 10.08; O, 9.37, according to these values, the molecular formula fits into the range $C_{22-23}H_{32-34}O_2$. The specific rotation was found to be $[\alpha]_D^{29} = +39.6^\circ$.

Unfortunately all the 23 mg. of the sterol stated had been used up and therefore further investigations could not be carried out.

Isolation of Lavandula Sterol B (Group III).—The fractions of Group III from Exp. II were mixed together by dissolving in ethyl acetate (8.4699 g.). Ethyl acetate was removed completely and the remaining oil was dissolved in a few ml. of petroleum ether, and kept for crystallization. Nothing came out. Petroleum ether was then completely removed and ethyl alcohol was added when some turbidity occurred. On keeping for about a fortnight needle-shaped crystals started settling down, but in a very small amount. It was therefore cooled in a refrigerator when more of the crystals came out. It was then sucked off and dried over phosphorus pentoxide. Yield, 0.25 g.; m.p. 123-126°C.

The mother liquor was concentrated to about 10 ml. and again kept in a refrigerator. Crystals again started depositing. Yield, 0.123 g.; m.p., 124-127°C.

The I and II crops were mixed together (wt. 0.373 g.) and recrystallised from petroleum ether. Rod-shaped crystals deposited. Yield 0.16 g., m.p. 135-136°C. On three more recrystallization the m.p. was constant at 136-137°C., $[\alpha]^{24} = -23.5^\circ$. Found: C, 83.20; H, 11.86; O, 4.94 by difference). According to the values given (the molecular formulae fits into the range: $C_{22}H_{36-38}O$. The substance gives positive Liebermann and digitonin tests.

Isolation of Lavandula Sterol C.—The substance which was sparingly soluble in petroleum ether and settled down on concentration of the petroleum ether extracts was named lavandula sterol C.

PURIFICATION.—Various experiments were carried out to purify the substance as it was contaminated with chlorophyll. Among them the soxhlet extraction with chloroform proved to be the best.

The raw substance (8.905 g.) was brought into a thimble of filter paper and soxhleted with chloroform for 6½ hours. The extracts previously dark green remained nearly colourless. The receiver was taken out and the solvent renewed. The dark green first extract was allowed to stand overnight when a lot of solid substance was observed to be floating on top of the solution. It was sucked off and washed four times with chloroform, pressed on a porous plate and weighed. Yield, 2.274 g. The mother liquor on further concentration did not yield more of the white substance. Brought to dryness, a solid dark green mass was obtained which is still under investigation.

The substance was now completely white but amorphous. At about 224°C. shrinking starts, at 240°C. it becomes brown, and melts at 247-249°C. with decomposition. The melt solidified to a brown lacquer. No sublimation could be observed. The substance was soluble in pyridine and tetrahydrofuran; sparingly in benzene, chloroform, ether, petroleum ether and ethanol; and insoluble in water and cyclohexane. It was recrystallized from ethanol.

RECRYSTALLIZATION.—The substance (1.10 g.) was dissolved in ethanol by heating on a water-bath and after filtration was kept for crystallization. Needle-shaped crystals started coming out on standing for some time. They were sucked off on a sintered glass crucible and dried in vacuum over phosphorus pentoxide. After two further recrystallizations the melting point was constant at 268-270°C.

Found: C, 77.42; H, 10.58; O, 12.46, which fits into the following molecular formulae: $C_{26}H_{40}O_3$

requiring C, 77.95; H, 10.07; O, 11.98. $C_{26}H_{42}O_3$
 requiring C, 77.56; H, 10.62; O, 11.86. $C_{26}H_{44}O_3$
 requiring C, 77.17; H, 10.96; O, 11.86.

The substance gives positive Liebermann-Burchard test for sterol. The digitonin test however was negative.

Acetyl Derivative of Lavandula Sterol C.—The substance (200 mg.) with 4 ml. of acetic anhydride and three drops of pyridine was refluxed for five hours at 140–150°C. on a glycerine bath. The substance completely dissolved in acetic anhydride on heating. Nothing came out on cooling. The mixture of acetic anhydride and acetic acid was removed under reduced pressure and water added. The outgoing substance was sucked off and dried over phosphorus pentoxide. Yield, 0.298 g.; m.p., 256–262°C.

SOLUBILITIES.—Easily soluble: tetrahydrofuran, glacial acetic acid (hot), alcohol (hot). Sparingly soluble: petroleum ether, ethyl acetate, chloroform, carbon tetrachloride, dioxane, dimethyl formamide, alcohol (cold), acetone, acetic acid. Insoluble: water. Recrystallisable: glacial acetic acid-water mixture.

The acetyl derivative was dissolved in glacial acetic acid and precipitated with water. After three repeated recrystallisations the m.p. was constant at 278–280°C., $[\alpha]_D^{26} = +65^\circ$ in 1% tetrahydrofuran solution, the acetyl value was found to be 8.96%. $C_{26}H_{42}O_2 \cdot O \cdot CO \cdot CH_3$ (444) requires: 9.6% $-CO \cdot CH_3$.

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