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TERPENOIDS—XLVI

COMPONENTS OF INDIAN VALERIAN ROOT OIL*

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Abstract—Indian valerian root oil obtained from the roots of the plant *V. wallichi* collected from different areas has been examined. Neutral portion of the oil contains ar-curcumene, α,β,γ-patchoulenes, patchouli alcohol, β-sitosterol, valeranone, maali-oxide, maaliol, calarene, and the new sesquiterpene hydrocarbon β-bergamotene which is an isoprenologue of β-pinene. Acidic portion of the oil is composed of formic, propionic, butyric, isovaleric, β-methyl valeric, palmitic, steric acids, and isovaleryl ester of α-hydroxyisovaleric acid along with some unidentified acids.

WHILE the components of the roots of the Continental variety of valerian (*Valeriana officinalis*) have been systematically studied,[1-3] very little is known about the constituents of the Indian variety (*V. wallichi* DC). Most of the work carried out so far is limited to the physico-chemical properties of the oil, general classification of the constituents which had only been obtained in impure form.[4-7] A more critical examination has been carried out and the results reported in this communication.

Based on the experience on costus root oil,[8] the constituents of the roots were initially collected by low temperature solvent extraction procedure generally employed for isolation of terpenic constituents.

The powdered roots were thoroughly extracted with light petroleum (40–60°) at room temperature, the solvent removed *in vacuo* at a bath temperature of 40° and the resulting thick extractive taken up in alcohol. On prolonged cooling of the alcoholic solution at 0° a waxy material separated which was filtered off. The alcohol was then removed and the resulting absolute distilled *in vacuo* initially using a rotary pump and finally a diffusion pump and separated into four main fractions as shown in the chart given below. There are two types of valerian roots available in India, viz., (i) roots with rootlets and (ii) roots without rootlets. Both the varieties have been

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* Contribution No. 652 from the National Chemical Laboratory, Poona-8, India.

[1] J. Kripinsky, V. Herout and F. Šorm, *Coll. Czech. Chem. Comm.* **24**, 1884 (1959).

[2] A. Stoll and E. Seebeck, *Liebigs Ann.* **603**, 158 (1957).

[3] A. Stoll, E. Seebeck and D. Stauffacher, *Helv. Chim. Acta* **40**, 1205 (1957).

[4] Tejsingh, Viswapal and K. L. Handa, *Ind. Perf.* **1**, 55 (1957).

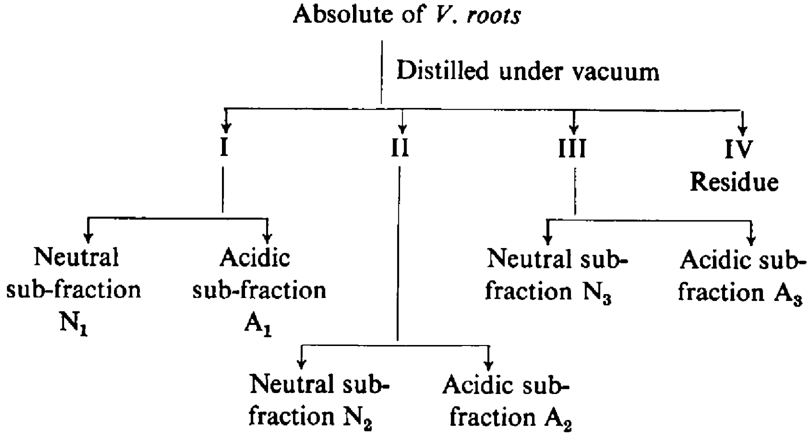
[5] Tejsingh and K. L. Handa, *Ind. Oil and Soap J.* **25**, 178 (1959).

[6] K. Bullock, *Pharm. J.* **117**, 152 (1926).

[7] Sadgopal and B. C. Gulati, *Soap Perf. Cos.* **28**, 1006; 1129; 1261 (1956).

[8] A. Paul, A. S. Bawdekar, R. S. Joshi, G. H. Kulkarni, A. S. Rao, G. R. Kelkar and S. C. Bhattacharyya, *Perf. & Ess. Oil Rec.* **51**, 115 (1960).

examined in the present investigation. The results of our investigation of roots with rootlets are described first.



The first three fractions mentioned were separated into neutral and acidic sub-fractions and each was examined separately.

The neutral sub-fraction N_1 on cooling at 0° for a prolonged period gave a crystalline alcohol, m.p. 104°, identified as maaliol* (I) from its physical properties, mixed m.p. and comparative IR spectra.[9] The residual material on extensive chromatography in stages afforded along with other products two hydrocarbons (A) and (B) in pure form (GLC) as the major constituents, both having the formula $C_{15}H_{24}$.

The hydrocarbon (A) which was eluted first contained one double bond and was therefore tricyclic. From an examination of its properties including NMR data, which indicated the presence of a cyclopropane ring and one olefinic proton, it has been found to be identical with calarene (II).[10–12]

The hydrocarbon (B) showed the presence of two double bonds. Chemical evidence and IR spectrum indicated that one of its double bonds was exocyclic (3030, 1745, 1653, 874 cm^{-1}) and the other tri-substituted (832 cm^{-1}) in the form of isopropylidene group (single peak at 1370 cm^{-1}). Further systematic degradation to β-pinene skeleton showed it to be a new sesquiterpene hydrocarbon of the β-pinene group represented by the structure III. This has been confirmed by comparative NMR study of this compound and its tetrahydro derivative along with those of α-santalene, β-santalene and their corresponding hydrogenated products. As its α-analogue (IV) has been previously obtained by Šorm[13] and named by him as bergamotene, we

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* We are indebted to Prof. G. Buchi for taking mixed m.p.s with authentic samples of maaliol and maalioxide.

[9] G. Buchi, M. S. Wittenau and D. M. White, *J. Amer. Chem. Soc.* **81**, 1968 (1959).

[10] F. Šorm, M. Holub, V. Sykora, J. Mleziva, M. Streibl, J. Pliva, B. Schneider and V. Herout, *Coll. Czech. Chem. Comm.* **18**, 512 (1953).

[11] G. Buchi, F. Greuter and T. Tokroyama, *Tetrahedron Letters* No. 18, 827 (1962).

[12] In a recent communication (*Tetrahedron Letters*, No. 4, 225 (1963)) Šorm and collaborators have shown that calarene is identical with β-gurjunene.

[13] V. Herout, V. Ruzicka, M. Vrany and F. Šorm, *Coll. Czech. Chem. Comm.* **15**, 373 (1950).

propose that the compound obtained by us be named as β-bergamotene[14] and that of Prof. Šorm as α-bergamotene in analogy with α- and β-pinene.

From the neutral sub-fraction N_2 three compounds have been so far isolated. One of these has been identified as valeranone[15] (V) from its IR spectrum, m.p. and mixed m.p. of its semicarbazone.* The second product is a crystalline oxide, m.p. 66°, and is identical with maalioxide (VI) previously obtained by Buchi[9] by treatment of maaliol with iodine. This is probably the first instance of the occurrence of maalioxide in nature.

The neutral fraction N_3 was found to contain three hydrocarbons in addition to a naphthalenic hydrocarbon which was isolated initially by chromatography and finally as the TNB complex, m.p. 133–134°.

From thin layer, paper and gas liquid chromatography the acidic fractions A_1, A_2 and A_3 were found to contain isovaleric acid and dextrorotatory β-methylvaleric acid as the main components, along with formic, propionic, butyric, palmitic, stearic acids and isovaleryl ester of D(−)-α-hydroxyisovaleric acid.[16] An unsaturated acid, $C_{12}H_{22}O_2$, has also been isolated.

Among the products isolated from the oil from roots with rootlets collected from an adjoining area, isolation of β-sitosterol along with substantial quantities of patchouli alcohol[17] and small amounts of α, β- and γ-patchaulenes (VIII, IX and X) is worth mentioning. The same sample of oil contained traces of maaliol, but no maalioxide.

By employing a procedure similar to one mentioned above, oil from roots without rootlets has been found so far to contain ar-curcumene, β-bergamotene, valeranone, maalioxide, maaliol, some unidentified sesquiterpenes together with almost the same acids as in the previous case.

EXPERIMENTAL

M.p.s are uncorrected. Specific rotations were determined in chloroform solution unless otherwise specified. UV absorption spectra were measured in ethanol with a Beckman Model DK-2 spectrophotometer. IR spectra were taken in a Perkin-Elmer (Model 137b) infracord spectrophotometer.

Paper chromatography was done in a system butanol (40 ml) ethanol (10 ml) and water (50 ml) and 4 ml liquor ammonia being added to the organic layer. The substances were added as ammonium salts. The chromatograms were sprayed with bromophenol blue. The reversed phase chromatography was done according to the methods of Kaufmann and Nitsch.[18]

GLC was done in squalane and carbowax columns.

Extraction of oil. To the powdered roots (36 kg) light petroleum (40–60°; 90 l.) was added and the mixture stirred by a spark-proof motor stirrer for 2 hr. After standing (15 min) the extract was filtered. This operation was repeated twice with 54 l. light petroleum each time. From the combined filtrates, the solvent was removed at 40° (red. press.), 72 kg of roots gave 1·68 kg of extractive.

The entire extractive was dissolved in alcohol (10 l.) and the solution kept at 0° for 24 hr. The wax that separated was filtered and washed with ice cold alcohol. The combined filtrates and washings were kept at 0° again for 24 hr and wax removed as before. From the final filtrate alcohol was removed (red. press.) at 60°. The absolute obtained was 1·5 kg.

Fractionation of the oil. Fractionation was done in small lots and appropriate fractions were combined; 2·6 kg of oil, gave 1·025 kg of fraction (I), b.p. 45–96°/5 mm, n_D^{29} 1·4270, $(\alpha)_D$ +6·47°

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[*] We are thankful to Prof. T. R. Govindachari for a sample of the semicarbazone.

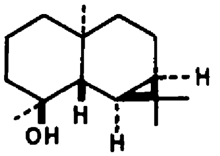
[14] K. S. Kulkarni, S. K. Paknikar, A. S. Vaidya, G. R. Kelkar, R. B. Bates and S. C. Bhattacharyya, *Tetrahedron Letters* No. 8, 505 (1963).

[15] J. Kripinsky, M. Romanuk, V. Herout and F. Šorm, *Coll. Czech. Chem. Comm.* **27**, 2638 (1962) and references cited therein.

[16] M. E. Cionga, *C. R. Acad. Sci., Paris*, **201**, 1152 (1935).

[17] G. Buchi and R. E. Erickson, *J. Amer. Chem. Soc.* **78**, 1262 (1956).

[18] H. P. Kaufmann and W. H. Nitsch, *Fette Seifen Anstrichmittel* **56**, 154 (1954).



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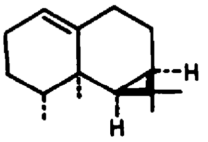

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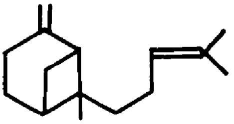

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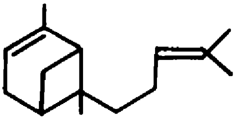

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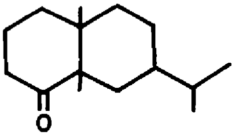

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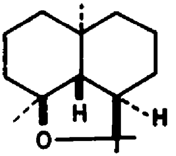

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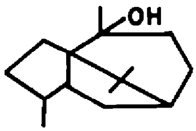

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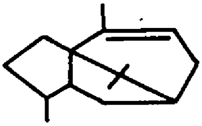

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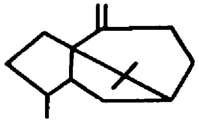

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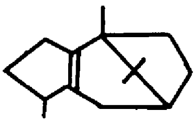

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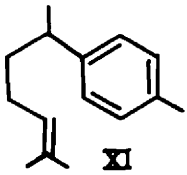

XI

(c, 4·0); acid value 283·6; sap. value 32·3; 480 g fraction (II), b.p. 44–147°/0·1 mm, n_D^{29} 1·4410, $(\alpha)_D$ +1·2° (c, 5·0), acid value 242·5 and 292 g fraction (III), b.p. 91–180°/0·08 mm, n_D^{31} 1·4325, acid value 217·4; sap. value 66·0. A thick greenish brown residue was left behind.

The above 3 fractions were then dissolved in ether and separated into acidic and neutral fractions by treatment with 10% NaOH aq. followed by the usual operations to give the acidic fractions, A_1, 524 g, A_2 385 g, A_3, 157 g, and the neutral fractions N_1, 252 g, N_2, 74·45 g and N_3, 52 g respectively.

Isolation of maaliol (I). The neutral portion N_1 was kept at 0° for a fortnight when maaliol separated out. It was sublimed (red. press.) m.p. 104° $(\alpha)_D$ +38·7° (c, 4·0), +21·7° (alcohol c, 5·0). It was identified by mixed m.p. with an authentic sample. The IR spectrum agreed with that of maaliol given in literature.[19]

Isolation of calarene (II). The mother-liquor (230 g) after separation of maaliol was chromatographed on alumina (grade II, 1:30) and 2 main fractions were collected; (a) light petroleum fraction (180 g) and (b) ether fraction (2·5 g).

The light petroleum fraction was rechromatographed on alkaline alumina (grade I, 1:30) and 14 × 250 ml light petroleum fractions collected. Fractions 3–9 were mixed together (106 g) based on rotation and refractive index values and passed through alkaline alumina (grade I, 1:100) and 28 × 250 ml light petroleum fractions were collected. Fractions 1–5 were mixed together. On evaporation of solvent calarene (5 g) was obtained; n_D^{28} 1·5031, $(\alpha)_D$ +44·5° (clean), (Found: C, 88·16; H, 12·13. $C_{15}H_{24}$ requires: C, 88·16; H, 11·84%).

IR was found to be identical with that described in literature.[10]

Isolation of β-bergamotene (III). Fractions 6–28 of the above chromatography were mixed together (47·15 g) and again rechromatographed on alkaline alumina (grade I, 1:60) and 25 × 50 ml light

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[19] J. Pliva, M. Horak, V. Herout and F. Šorm, *The Terpenes, Coll. of Spectra and Physical Constants* **1**, S 199 (1960).

petroleum fractions were collected. Fraction 25 was rich in β-bergamotene (GLC).* This fraction was rechromatographed over alumina (grade I, 1:300) to give pure β-bergamotene, b.p. 120–130° (bath)/1 mm, n_D^{27} 1·4949, d_4^{27} 0·8841, $(\alpha)_D$ +35·8° (c, 4·0).

Neutral fraction N_2

Isolation of valeranone and maalioxide. The fraction (74 g) was chromatographed on neutral alumina (grade II 1:30) and fractions collected as follows: Light petroleum (6 l.); light petroleum + benzene (5 l.); benzene (2 l.); and alcohol (1 l.). The light petroleum fraction contained calarene and β-bergamotene.

IR spectrum of light petroleum–benzene fraction indicated the presence of a carbonyl compound which was separated by treatment with Girad's reagent T. The separated ketone was identified as valeranone through its semicarbazone, m.p. and mixed m.p. 205° (DNP m.p. 102·5°). The ketone regenerated from semicarbazone (oxalic acid–hexane) had the following properties: b.p. 140°(bath)/2 mm, n_D^{25} 1·4917; $(\alpha)_D$ −55·12° (c, 5·3) (Found: C, 81·45; H, 12·34. $C_{15}H_{26}O$ requires: C, 81·02; H, 11·79%). The non-ketonic fraction solidified on keeping at 0° for several days. It was sublimed under vacuum and identified as maalioxide, m.p. and mixed m.p. with a sample of maalioxide prepared from maaliol[9] was 65° $(\alpha)_D$ +33·28° (c, 4·0) (Found: C, 81·08; H, 11·87; $C_{15}H_{26}O$ requires: C, 81·02; H, 11·79%). Besides maalioxide a fraction showing the presence of acetate group was isolated. This has not been further investigated.

Neutral fraction N_3

From column chromatography and GLC analysis, this fraction was found to be composed of 3 sesquiterpene hydrocarbons ($C_{15}H_{24}$). The individual components however could not be obtained in pure form so far.

Later fractions having higher refractive indices were found to contain a naphthalenic product (UV) which yielded a sym. TNB complex m.p. 133–134°.

Acid portion of oil from roots with rootlets. The acid fraction A_1 (183 g) was fractionated *in vacuo* using a Tower's column wherever necessary and divided into about 20 fractions each of which was examined for physical properties, equiv. wt and R_f value and was found to be composed essentially of isovaleric acid (R_f 0·6) and dextrorotatory β-methylvaleric acid (R_f 0·69) both of which could be isolated on a preparative scale. β-Methylvaleric acid was further confirmed by its optical rotation $(\alpha)_D$ +7·4° (clean), anilide m.p. 85° and amide m.p. 123°.

The acid fraction A_2 (170 g) was similarly divided into 11 fractions by vacuum fractionation. Paper chromatography and GLC analysis showed fractions 1–4 to be composed essentially of isovaleric and β-methylvaleric acids. Fractions 9–11 gave solids which were identified as stearic and palmitic acids by reversed phase paper chromatography. Intermediate fractions 5 to 8 (28 g) showed spots in paper chromatography corresponding to more polar compounds. These were combined and fractionated *in vacuo* (1 mm) in a spinning band column and sub-divided into several fractions. Fractions 1–6 were composed of isovaleric and β-methylvaleric acids. Fractions 7–12 showed spots at 0·43, 0·58 and 0·73 R_f values and could be possibly attributed to new acids.

Fractions 8 and 9 (0·355 g) both having the same R_f value (0·43) and equiv. wt 197 were combined and distilled, 130° (bath)/2 mm. (Found: C, 72·01; H, 10·71. $C_{12}H_{22}O_2$ requires: C, 72·68; H, 11·18%). The IR spectrum indicated the presence of double bond and absence of any conjugation (UV).

Acid fraction A_3 (100 g) was subdivided into 14 fractions (58–138°/35 mm) through Tower's column. Paper and GLC chromatography indicated that fractions 1–5 were composed of isovaleric and β-methylvaleric acids (R_f 0·60 and 0·68 respectively). Fractions 6–9 contains a highly polar acid (R_f 0·47) besides valeric and β-methylvaleric acids and fractions 10–14 contain a different acid (R_f 0·74), b.p. 136°/35 mm.

A small amount of this acid after saponification and usual processing gave two distinct acids on fractional distillation. The lower boiling acid was identified as isovaleric acid. The higher boiling component was found to be D-(−)-α-hydroxyisovaleric acid,[10] b.p. 138–142°/30 mm, which solidified

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* We are thankful to Dr. V. G. Naik for the GLC analysis.

[10] L. C. Vining and W. A. Taber, *Canad. J. Chem.* **35**, 1109 (1957).

on keeping and purified by sublimation, m.p. 62–63° $(\alpha)_D$ −15° (*c*, 2·19; lit. 19·1°). (Found: C, 50·5; H, 8·7. Equiv. wt. 122·5. $C_5H_{10}O_3$ requires: C, 50·83; H, 8·53%).

Hence this acid is the isovaleryl ester of D(−)-α-hydroxyisovaleric acid.

Isolation of patchouli alcohol (VII). Valerian root oil (2·1 kg) from roots with rootlets from another locality was used without fractionation and separated into neutral and acid portions. The neutral fraction (350 g) was chromatographed in batches over alumina (grade III, 1:30) and 3 major fractions were collected (a) light petroleum 137 g (b) ether, 117 g and (c) alcohol 15 g.

Major fraction 'a' was chromatographed over neutral alumina (grade II, 1:30) and 17 fractions were collected in the following order, 1–8 light petroleum; 9–15 benzene; and 16–17 ether. Fraction 15 solidified on keeping, the solid purified by crystallization and sublimation m.p. and mixed m.p. 57° $(\alpha)_D$ −121·8° (*c*, 0·8) (Found: C, 81·0; H, 11·7. $C_{15}H_{26}O$ requires: C, 81·02; H, 11·79%). IR spectrum and all its properties were identical with that of patchouli alcohol.[21]

Isolation of β-patchoulene (IX) *and β-sitosterol*. The ether fraction of the above chromatography (major fraction 'b') was chromatographed on neutral alumina (grade II, 1:30) and eluted with light petroleum, benzene and ether respectively.

The first 2 light petroleum fractions were combined and rechromatographed on alumina (grade I, 1:150) and 14 fractions were collected by elution with light petroleum only. The first fraction showed a single peak in GLC while the second fraction showed one major and one minor peak.

The first fraction was distilled over sodium (Found: C, 87·86; H, 11·71. $C_{15}H_{24}$ requires: C, 88·16; H, 11·84%). IR and NMR spectra confirmed its identity with β-patchoulene.[17] Fractions 2 and 3 were constituted of α-, β-, and α-, β- and γ-patchoulenes respectively, confirmed through comparative GLC analysis with mixture of patchoulenes prepared from patchouli alcohol.

Addition of 90% methanol to the ether fraction of the above chromatography precipitated a solid, identified as β-sitosterol, m.p. and mixed m.p. 137°.

IR spectrum was identical with that of authentic sample.

Oil from roots without rootlets. The oil (1·38 kg) from 162 kg roots was separated as before into acidic fractions a_1 (285 g), a_2 (98 g) and a_3 (93 g) and neutral fractions n_1 (81 g), n_2 (45 g) and n_3 (13 g).

The acidic fractions were found to contain essentially the same acids as described before. The neutral fraction n_1 on chromatography and GLC analysis was found to contain calarene, β-bergamotene and ar-curcumene. Fraction n_2 was constituted of ar-curcumene, valeranone, maalioxide and maaliol. ar-Cureumene isolated from fraction n_2 had the following properties, b.p. 98°/2 mm, n_D^{29} 1·5000, $(\alpha)_D$ +36·56° (*c*, 1·4) (Found: C, 88·94; H, 11·2. $C_{15}H_{22}$ requires: C, 89·04; H, 10·96%).

Fraction n_3 has not yet been examined.

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* Microanalysis were carried out by Pansare and colleagues. Spectral measurements were carried out by H. Gopinath and K. G. Deshpande.

[21] Ref. 19, plate No. S217.