## 75. *The Triterpene Group. Part XI. The Non-Saponifiable Matter of Lactucarium Germanicum.*

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The non-saponifiable matter of Lactucarium germanicum has been examined. It is shown that the conflicting results of earlier workers are due to failure to isolate pure constituents from the complex mixture of triterpene alcohols present in the latex. Three of these alcohols have now been obtained in a state of purity, namely, taraxasterol, β-amyrin, and a new monohydric alcohol, *germanicol*, of probable formula $C_{30}H_{50}O$.

Lactucarium germanicum, the dried latex of *Lactuca virosa*, contains a mixture of crystalline esters. Many attempts have been made by various workers to isolate the esters or the related alcohols in a state of purity, with conspicuous lack of success. Following the earliest memoirs of a century ago, investigations have been carried out by, amongst others, Hesse (*Annalen*, 1886, **234**, 243; 1888, **244**, 268), Kassner (*ibid.*, 1887, **238**, 220), Pomeranz and Sperling (*Monatsh.*, 1904, **25**, 785), and Bauer and Schub (*Arch. Pharm.*, 1929, **267**, 413), and Zellner and his pupils (*Monatsh.*, 1926, **47**, 681) have examined the latex of the closely related *Lactuca sativa*. Each of the authors named appears to have believed that a successful isolation of pure constituents had been accomplished, but a comparison of the published results (Tables I and II) shows this to be untrue, because, although some measure of general agreement is apparent, discrepancies in detail are abundant. The

TABLE I.

*Sources and Constants of "α-Lactucerol."*

| Genus and species. | Alcohol (Calc. for $C_{30}H_{50}O$: C, 84·4; H, 11·8%). M. p. | $[\alpha]_D$. | % C. | % H. | Acetate (Calc. for $C_{32}H_{52}O_2$: C, 82·0; H, 11·2%). M. p. | $[\alpha]_D$. | % C. | % H. | Benzoate (Calc. for $C_{37}H_{54}O_2$: C, 83·8; H, 10·3%). M. p. | $[\alpha]_D$. | % C. | % H. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 179° | +76° | 82·4 | 11·4 | 210° | 63° | 79·1 | 10·7 | 156° | — | 81·8 | 10·4 |
| 2 | No alcohol resembling α-lactucerol obtained | | | | | | | | | | | |
| 3 | 194 | 52 | 83·0 | 11·5 | 215 | — | 80·6 | 11·1 | — | — | — | — |
| 4 | 217 | 97 | 83·7 | 11·7 | 250 | — | 81·5 | 11·2 | 257 | — | 83·5 | 10·3 |
| 5 | 180 | — | 82·1 | 11·3 | — | — | — | — | — | — | — | — |
| 6 | 197 | — | 83·8 | 12·0 | 220 | — | — | — | — | — | — | — |
| 7 | 197 | — | — | — | 220 | — | 81·6 | 10·9 | — | — | — | — |
| 8 | 199 | — | — | — | — | — | — | — | — | — | — | — |
| 9 | 206 | 94·5 | 83·5 | 11·7 | 220 | — | 80·6 | 11·6 | — | — | — | — |
| 10 | No data given for α-lactucerol | | | | | | | | | | | |
| 11 | 203 | 78·4 | 84·2 | 11·7 | — | — | — | — | 257 | — | 83·4 | 10·6 |
| 12 *ca.* | 179 | 50 | — | — | 210 | 73 | 80·6 | 11·1 | — | — | — | — |
| 13 * | 224 | 89 | 84·1 | 11·9 | 239 | — | 82·3 | 11·2 | 255—257 | — | 83·7 | 10·3 |

TABLE II.

*Sources and Constants of "β-Lactucerol."*

| Genus and species. | Alcohol (Calc. for $C_{30}H_{50}O$: C, 84·4; H, 11·8%). M. p. | $[\alpha]_D$. | % C. | % H. | Acetate (Calc. for $C_{32}H_{52}O_2$: C, 82·0; H, 11·2%). M. p. | $[\alpha]_D$. | % C. | % H. | Benzoate (Calc. for $C_{37}H_{54}O_2$: C, 83·8; H, 10·3%). M. p. | $[\alpha]_D$. | % C. | % H. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | — | 38° | 82·5 | 11·6 | 230° | — | 78·9 | 10·5 | — | — | — | — |
| 2 | 154° | — | 83·4 | 11·3 | 184 | 50° | 80·2 | 10·5 | — | — | — | — |
| 3 | 150 | — | 83·4 | 12·1 | 228 | — | — | — | — | — | — | — |
| 4 | 155 | — | 83·3 | 11·6 | 210 | — | — | — | — | — | — | — |
| 5 | 150 | 48·5 | 83·8 | 12·2 | — | — | — | — | — | — | — | — |
| 6 | 150 | — | 84·2 | 12·1 | — | — | — | — | — | — | — | — |
| 7 | 150 | — | 84·3 | 11·7 | 285 | — | — | — | — | — | — | — |
| 8 | 155 | — | — | — | — | — | — | — | — | — | — | — |
| 9 | 152 | 49 | 83·8 | 12·8 | 282 | — | 82·5 | 11·2 | — | — | — | — |
| 10 | 154 | — | 84·3 | 11·5 | — | — | — | — | — | — | — | — |
| 11 | 165 | 54 | 84·3 | 12·1 | — | — | — | — | 260° | — | 83·8 | 10·4 |
| 12 | No data given for β-lactucerol | | | | | | | | | | | |
| 13 * | 178—180 | 51 | 84·0 | 11·8 | 232 | — | 81·9 | 11·7 | 222—224 | — | 83·7 | 10·3 |

1 *Lactuca virosa*; Hesse, *loc. cit.*
2 *L. virosa*; Pomeranz and Sperling, *loc. cit.*
3 *Sonchus arvensis*; Stern and Zellner, *loc. cit.*
4 *Taraxacum officinale*; Zellner and co-workers, *loc. cit.*
5 *L. sativa*; *idem, ibid.*
6 *S. asper*; *idem, ibid.*
7 *Trapogon pratensis*; *idem, ibid.*
8 *Cichorium endivia*; *idem, ibid.*
9 *C. intybus*; *idem, ibid.*
10 *Scorzonera hispanicus*; *idem, ibid.*
11 *L. virosa*; Bauer and Schub, *loc. cit.*
12 *L. virosa*; Kassner, *loc. cit.*
13 *L. virosa*; Bauer and Brunner, *Arch. Pharm.*, 1938, **276**, 605.

TABLE III.

*Sources and Constants of Taraxasterol.*

| Genus and species. | Alcohol, $C_{30}H_{50}O$. M. p. | $[\alpha]_D$. | % C. | % H. | Acetate, $C_{32}H_{52}O_2$. M. p. | $[\alpha]_D$. | % C. | % H. | Benzoate, $C_{37}H_{54}O_2$. M. p. | $[\alpha]_D$. | % C. | % H. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 221—222° | +96° | 84·5 | 11·7 | 251—252° | 102·5° | 81·9 | 10·9 | 232° | — | 83·2 | 10·0 |
| 2 | 221—222 | 96 | 84·5 | 11·7 | 251—252 | 100·5 | 82·1 | 11·7 | 242—244 | 107° | 83·5 | 10·1 |
| 3 | 217—219 | 96 | 84·4 | 11·7 | 248—250 | 99 | 81·7 | 11·2 | — | — | — | — |
| 4 | 220—221 | — | — | — | 250—252 | 101 | — | — | — | — | — | — |
| 5 | 221—222 | 97 | 84·6 | 11·6 | 250—251 | 97 | — | — | 242—244 | 105 | — | — |

1 *Taraxacum officinale*; Power and Browning, *loc. cit.*
2 *Taraxacum officinale*; Burrows and Simpson, *loc. cit.*
3 *Anthemis nobilis*; Power and Browning, *loc. cit.*
4 *Anthemis nobilis*; Burrows and Simpson, *loc. cit.*
5 *Lactuca virosa*; this paper.

* The literature regarding these alcohols is further complicated by the fact that Bauer and Brunner claim to have isolated lactucadiene, $C_{30}H_{48}$, m. p. 154°, from both "α"- and "β-lactucerol." As the ketones derived from each alcohol are not identical, this claim involves a novel relationship between the two alcohols; however, in view of the work described in the present paper, Bauer and Brunner's claim should be accepted with the greatest reserve, as they have not established the homogeneity of "β-lactucerol," and its contamination with taraxasterol seems to be extremely likely. A recent publication by Hesse, Eilbracht, and Reichender, of which only the abstract (*A.*, 1942, II, 43) is available, states that "α-lactucerol" from *Calotropis* resin is identical with taraxasterol. The authors regard it as a *primary* alcohol, but state that it gives sapotalin on dehydrogenation and that treatment with acids converts it into "*iso*lactucerol." This substance may conceivably be a slightly impure specimen of ψ-taraxasterol (Burrows and Simpson, *loc. cit.*; Morice and Simpson, J., 1940, 795), particularly as Murti and Seshadri (*A.*, 1944, II, 110), working with *Calotropis gigantea*, isolated a substance named β-calotropeol. Dr. Murti's thesis has just been in the present author's hands, and, short of confirmation by mixed melting points, the identity of β-calotropeol with ψ-taraxasterol is highly probable. Our experience with ψ-taraxasterol has shown that its complete separation from taraxasterol is almost impossible, and the isomerisation recorded by Hesse *et al.* may not have been quantitative.

only facts beyond dispute are that the latex contains a mixture of high-melting dextrorotatory acetates (known variously as lactucerin and lactucon), and that on hydrolysis these yield crystalline dextrorotatory alcohols of uncertain composition, to which the names " α- and β-lactucerol " (or " -lactucol ") have been ascribed.

Despite the fact that the properties of these alcohols depend markedly on the source from which they are prepared, Zellner (*loc. cit.*; also Stern and Zellner, *ibid*, 1925, **46**, 458) has assumed that " α- " and " β-lactucerol " are two homogeneous entities, and has sought to show that they are characteristic constituents of various species of *Compositæ* (of which *Lactuca* is one genus). In addition, he has claimed that " α-lactucerol " is identical with the triterpene alcohol taraxasterol, which has been isolated in a state of undoubted purity from two *Compositæ* species, namely, *Taraxacum officinale* (Power and Browning, J., 1912, **101**, 2411; Burrows and Simpson, J., 1938, 2042) and *Anthemis nobilis* (Power and Browning, J., 1914, **105**, 1829; Burrows and Simpson, *loc. cit.*). A comparison of Tables I, II, and III shows that, while the evidence on which Zellner's claims are based is inadequate and unconvincing (it appears to lack even the support of mixed melting point determinations), it is yet sufficiently suggestive to warrant a closer investigation of the *Compositæ* species in question.

The experiments now reported show that Lactucarium germanicum does in fact contain taraxasterol, although probably not as a major component. The non-saponifiable matter consists almost entirely of a mixture of crystalline alcohols, which, judged from their general properties, are undoubtedly of triterpenoid character. The mixture is excessively complex, and a meticulous examination of the small quantity available was not attempted. However, the presence of β-amyrin was established, and a new triterpene alcohol was isolated without much difficulty. This substance has been named *germanicol*, and appears to be present in fair quantity; it melts at 177°, has the formula $C_{30}H_{50}O$, is unsaturated to tetranitromethane, and gives a crystalline *acetate* (m. p. 274—276°) and *benzoate* (m. p. 270°).

The names lactucerin, lactucon, α- and β-lactucerol, and α- and β-lactucol must now be regarded as descriptive of mixtures. No fraction having a constant melting point similar to that of " β-lactucerol " was encountered, but a significant fact is that the published melting points of several preparations of " α-lactucerol " lie very close to the melting point (195—197°) of what was found, in the present work, to be a constant-melting mixture containing taraxasterol.

The difference between these results and those of earlier workers is believed to be due to experimental procedure and not to phyllogenetic causes. The inclusion of a stage involving adsorption on activated alumina appears to be an essential step; it has yielded good results in similar problems (*e.g.*, Burrows and Simpson, *loc. cit.*; Morice and Simpson, J., 1940, 795; 1941, 181; Barton and Jones, J., 1943, 599), and a careful re-examination of other *Compositæ* species by the chromatographic procedure would probably yield valuable and conclusive results. In addition, such work might well disclose a practicable source of taraxasterol, which is of particular interest in that its double bond is easily reducible (Burrows and Simpson, *loc. cit.*), a fact which, from analogy with lupeol and betulin, is suggestive of nuclear divergence from the amyrin type.

## Experimental.

Melting points are uncorrected. Specific rotations were determined in chloroform in a 1-dm. tube. The ligroin used had b. p. 40—60°.

*Preparation of Non-saponifiable Matter.*—Commercial Lactucarium germanicum of British origin (110 g.) was broken into pieces the size of a pea and left under ligroin (1000 c.c.) for a fortnight with occasional shaking. The liquid was decanted, and the residue left for 10 days with more ligroin (750 c.c.). Evaporation of each extract left a solid mass (40 g. and 3 g. respectively). The total ligroin-soluble material was refluxed for 4 hours with a solution of potassium hydroxide (50 g.) in water (50 c.c.), alcohol (700 c.c.), and benzene (150 c.c.). The solution, from which a little insoluble rubber-like material (subsequently discarded) had separated, was then concentrated, 500 c.c. of solvent being recovered. The residue was diluted with water to 2·5 l. and extracted with ether. A small amount (*ca.* 0·5 g.) of a silky crystalline substance, insoluble in dilute potassium hydroxide solution, in ether, and in chloroform, separated from the ethereal phase during the extraction. The filtered ethereal extract was washed twice with water, a gel forming in the aqueous phase during this process. The aqueous gel was re-extracted with ether, and the extract added to the main ethereal solution. Acidification of the alkaline solution gave a low-melting mixture of solid acids (4 g.). Evaporation of the washed and dried neutral fraction yielded an almost white crystalline mass (33 g.), easily soluble in benzene and sparingly soluble in ligroin.

*Preliminary Fractionation of Non-saponifiable Matter.*—A solution of the foregoing material (33 g.) in 800 c.c. of benzene–ligroin (5 : 3) was drawn through a column (80 × 2·8 cm.) of Merck's activated alumina prepared by suspending 400 g. in 800 c.c. of benzene–ligroin (1 : 1). The column was then washed with successive portions of solvent, which were worked up separately as follows :

| Fraction No. | Washing solvent. | Weight (g.). | Appearance. |
|---|---|---|---|
| 1 | Original filtrate | 1·2 | Yellow mobile oil; strong musty odour similar to that of original latex |
| 2 | Benzene–ligroin (800 c.c., 5 : 3) | 16·5 | Crystalline solid |
| 3 | Benzene–ligroin (800 c.c., 6 : 2) | 5·3 | Crystalline solid |
| 4 | Benzene–ligroin (800 c.c., 6 : 2) | 2·5 | Crystalline solid |
| 5 | Benzene–ligroin (800 c.c., 6 : 2) | 1·3 | Crystalline solid |
| 6 | Benzene (800 c.c.) | 0·5 | Yellow resin |
| 7 | Benzene (800 c.c.) | 0·2 | Yellow resin |
| 8 | Ether–benzene (800 c.c., 3 : 5) | 0·5 | Yellow resin |
| 9 | Ether–benzene (800 c.c., 1 : 1) | 0·7 | Yellow frothy mass |
| 10 | Ether (800 c.c.) | 1·1 | Partly crystalline |
| 11 | Ether (800 c.c.) | 0·2 | Yellow resin |
| 12 | Ether (1600 c.c.) | 0·2 | Yellow resin |
| 13 | Methanol–benzene *ad lib.* | 2·0 | Yellow resin |
| | | 32·2 | |

*Isolation of Taraxasterol.*—Fractions 3, 4, and 5 were separately crystallised from alcohol; each had roughly the same solubility, and the initial crop in each case melted at about 160°. After several further crystallisations the m. p. in each case rose to *ca.* 185—190°. These crops were combined (2·78 g.), and the crystallisations continued; a mixture was thus obtained which behaved as a pure substance, as it melted at 195—197° during three successive crystallisations. The final crop (0·73 g.) was acetylated (pyridine and acetic anhydride), as was also the material (1·42 g.) remaining in the mother-liquor from this and the two preceding crystallisations. The resultant acetates were separately crystallised from benzene–alcohol until the m. p., originally 215—225°, rose to 242—244°. At this point the crops were combined (0·42 g.) and crystallised from ethyl acetate; taraxasteryl acetate (0·25 g.) was thus obtained as irregular plates, m. p. 250—251°, $[\alpha]_D^{20°}$ +97° ($c$ = 2·10), which gave no depression when mixed with an authentic specimen (m. p. 250—252°, $[\alpha]_D$ +100·5°) obtained from *taraxacum* root.

Hydrolysis of the acetate (3% benzene–alcoholic potassium hydroxide) gave taraxasterol, which separated from alcohol in soft fluffy needles, m. p. 221—222°, $[\alpha]_D^{17°}$ +97° ($c$ = 1·65) (Found: C, 84·6; H, 11·6. Calc. for $C_{30}H_{50}O$: C, 84·4; H, 11·8%). No depression in m. p. was observed when this preparation was mixed with an authentic sample of m. p. 221—222° and $[\alpha]_D$ +96°. Benzoylation of the alcohol gave taraxasteryl benzoate, m. p. 242—244°, $[\alpha]_D^{19°}$ +105° ($c$ = 1·85), which showed no depression when mixed with authentic material (m. p. 242—244°, $[\alpha]_D$ +107°).

*Germanicyl Acetate.*—Fraction 2, which was extremely soluble in alcohol, yielded 10·5 g. (A), m. p. 145—150°, after a single crystallisation from ether–aqueous alcohol. This material was acetylated (15 c.c. of pyridine, 15 c.c. of acetic anhydride) at 100°, and the mixed acetates (m. p. 210—230°), isolated by dilution with water, were repeatedly crystallised from benzene–alcohol, and finally from ethyl acetate. *Germanicyl acetate*, which was sparingly soluble in this solvent, formed long, triangular, transparent laminæ, m. p. 274—276°, $[\alpha]_D^{20°}$ +18·1° ($c$ = 2·155), which gave a pale yellow colour with tetranitromethane in chloroform (Found: C, 82·1; H, 11·25. $C_{32}H_{52}O_2$ requires C, 82·0; H, 11·2%). The compound was also isolated in smaller quantity from the mother-liquor of (A) after acetylation; total yield, 1·25 g.

Hydrolysis of germanicyl acetate (4% benzene–alcoholic potassium hydroxide under reflux) yielded *germanicol*, which separated from aqueous alcohol in tufts of small prismatic needles, m. p. 176—177°, $[\alpha]_D^{17°}$ +5·8° ($c$ = 2·925) (Found: C, 84·0; H, 11·75. $C_{30}H_{50}O$ requires C, 84·4; H, 11·8%).

*Germanicyl benzoate*, prepared by benzoylation of the alcohol in pyridine at 100°, was very sparingly soluble in hot acetone, gave an approximately 2% solution in boiling ethyl acetate, and separated from benzene–alcohol in thin brittle plates, m. p. 269—270°, $[\alpha]_D^{19°}$ +39·0° ($c$ = 2·26) (Found: C, 84·2; H, 10·35. $C_{37}H_{54}O_2$ requires C, 83·8; H, 10·3%).

*Isolation of β-Amyrin.*—The material remaining in the earlier benzene–alcohol mother-liquors, from which germanicyl acetate was obtained, was collected and fractionally crystallised from ethyl acetate. After removal of a number of fractions which apparently consisted of impure germanicyl acetate, several crops of prismatic needles were obtained which melted at *ca.* 225—230° and showed no depression when mixed with β-amyrin acetate. A specimen of the same purity was also obtained by acetylation of the material remaining in the first mother-liquors from fractions 3, 4, and 5. As further purification of this crude acetate by crystallisation seemed hopeless, it (1·4 g.) was hydrolysed with 2½% alcoholic potassium hydroxide under reflux, and the product benzoylated in pyridine solution. The mixed benzoates, crystallised once from benzene–alcohol, had m. p. 238—241° and 220—227° on admixture with β-amyrin benzoate. Continued crystallisation from ethyl acetate gave 0·5 g. of moderately pure germanicyl benzoate (this was obtained pure by further crystallisation and identified by m. p., mixed m. p., and conversion into germanicol). Extensive fractional crystallisation of the more soluble benzoates, combined with fractional digestion with ether, finally yielded 280 mg. of β-amyrin benzoate, which crystallised in the thin rhombs characteristic of this substance; it had m. p. 229—232° alone and mixed with an authentic specimen, and $[\alpha]_D^{19°}$ +93° ($c$ = 2·92). Its identity was fully established by hydrolysis to the alcohol, m. p. 186·5—189°, $[\alpha]_D^{19°}$ +81° ($c$ = 1·01) (a mixture with β-amyrin, m. p. 189—192°, melted at 187—190°), and this was finally converted into the acetate (characteristic rods from benzene–alcohol), which had m. p. 238—239° alone and on admixture with authentic material; $[\alpha]_D^{19°}$ +81·5° ($c$ = 2·09). Owing to the difficulty attending its isolation, it was impossible to assess even approximately the β-amyrin content of the latex.

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