148. *The Triterpene Group. Part VII. The Minor Triterpenoid Constituents of* Manila elemi *Resin.*

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A new and standardised method is described for the preparation of breïn from *Manila elemi* resin, depending on fractional elution from activated alumina, followed by formylation. Two additional triterpene alcohols have been obtained, the isolation of which from this resin has not hitherto been reported; these are *maniladiol*, a new dihydric alcohol isomeric with breïn, and ψ-taraxasterol, previously isolated from *Taraxacum* root (Burrows and Simpson, J., 1938, 2042). It is probable that the latter alcohol does not occur as such in *Manila elemi* resin, but is produced during the working up of the resin by cyclisation of a tetracyclic isomeride.

It is well established that the principal triterpenes of *Manila elemi* resin are the amyrins and elemic acid. The occurrence of small amounts of three other crystalline substances, breïn, bryoidin, and breïdin, has been observed by various investigators (Baup, *Jahresber. Chem.*, 1851, **4**, 528; Flückiger, *N. Rep. Pharm.*, 1875, **24**, 220; Vesterberg, "Kemiska studier öfver några hartser," Upsala, 1890, p. 99; *Ber.*, 1906, **39**, 2467; Tschirch and Cremer, *Arch. Pharm.*, 1902, **240**, 293); of these compounds, the last two are of unknown

constitution, but as they are appreciably volatile and somewhat soluble in water they cannot be regarded as triterpenoid in character. A possible relationship between breïn and the amyrins, however, was recognised by Vesterberg (*loc. cit.*), who characterised the compound as a dihydric alcohol, $C_{30}H_{50}O_2$, a formula recently confirmed by Rollet (*Monatsh.*, 1929, **53—54**, 231) and by Mladenović (*ibid.*, 1937, **70**, 276). Work on the constitution of this alcohol has been greatly hampered by the scarcity of material and by the lack of a standardised method of preparation, as has been fully emphasised by previous workers (Vesterberg, Rollet, *locc. cit.*).

A comparatively successful separation of the complex mixture of non-saponifiable substances from the fat of dandelion root was recently achieved by the method of fractional elution from activated alumina (Burrows and Simpson, J., 1938, 2042), and a similar procedure has now provided a suitable means for the preparation of breïn from *Manila elemi* residues. We have evolved a standardised method capable of yielding breïn in quantity, although there is some overlap in adsorbability between the diol and the monohydric amyrins.

Our experiments have also disclosed the presence of two further triterpene alcohols in the resin. Vesterberg (*loc. cit.*) and Rollet (*loc. cit.*) both observed that acetylation of impure breïn yielded a diacetate of the same melting point as that obtained from the pure diol, but neither author appears to have hydrolysed the former diacetate. This we have now done, and find that the resultant alcohol invariably melts at 206—208°. Formylation of this impure breïn yielded *breïn diformate*, m. p. 221° (hydrolysis of which furnished pure breïn, m. p. 222°), together with an isomeric *diformate*, m. p. 192°. The diol derived from this new diformate, for which the name *maniladiol* is suggested, melted at 221°, and gave a crystalline *diacetate* and *dibenzoate*.

We have also obtained, by the formylation method, a monohydric alcohol, which proved to be identical with the ψ-taraxasterol originally isolated by Burrows and Simpson (*loc. cit.*) from *Taraxacum* root. In the present work the alcohol was obtained from a fraction of the resin which was very strongly adsorbed on alumina, and it could not be isolated from any of the more weakly adsorbed fractions, despite the comparative insolubility of the formate. This observation is at first sight anomalous in view of the molecular formula, $C_{30}H_{50}O$, of ψ-taraxasterol, because such an alcohol, *ceteris paribus*, should be less strongly adsorbed than either breïn or maniladiol. A feasible explanation of this discrepancy is provided by the assumption that ψ-taraxasterol does not exist as such in *Manila elemi* resin, but arises by cyclisation, during formylation, of a supposedly tetracyclic, doubly unsaturated, isomeride. This view is strongly supported by the results of acetylation of the fraction in question. ψ-Taraxasteryl acetate, which is sparingly soluble, could not be obtained from the mixture of acetates even after nucleation, but was isolated without difficulty after treatment of the acetates with formic acid. Although our efforts to isolate the unisomerised alcohol have so far been unsuccessful, we believe that the evidence which we have described, taken in conjunction with the known cyclisation of basseol to β-amyrin (Beynon, Heilbron, and Spring, J., 1937, 989) and of onocerol diacetate to the isomeride of m. p. 260° (Zimmermann, *Helv. Chim. Acta*, 1938, **21**, 853) by means of formic acid, points to the existence of a similar precursor * of ψ-taraxasterol in *Manila elemi* resin.

Experiments bearing on the constitution of breïn are in progress, upon which it is hoped to report shortly.

Experimental.

(Melting points are uncorrected. Specific rotations are in chloroform solution.)

Adsorption of Manila elemi *Residues.*—The starting material was a concentrated solution of the resin in 85% alcohol, from which the greater part of the amyrins had previously been removed by crystallisation. Most of the alcohol was distilled off under reduced pressure, and the viscous turbid residue was then dissolved in ether (3 vols.) and shaken repeatedly with 4% sodium hydroxide solution, which removed most of the elemic acid. After being washed with water, the ethereal solution was concentrated, and the residue distilled in steam until the distillate was clear and almost odourless. The non-volatile residue was again taken up in ether;

* (Note added in proof.) This precursor has now been isolated. Details will be published later.

the solution, after being thoroughly dried (sodium sulphate) and concentrated, yielded an orange-coloured resin, which partly crystallised on long standing.

After preliminary experiments, the following batch process, which gave reasonably consistent results (see Table), was adopted for adsorption of the resin on activated alumina. A solution of the resin (150 g.) in benzene (1500 c.c.) was filtered through a column (120 × 3 cm.) of alumina (750 g. of Merck's, " standardisiert nach Brockmann "), after which the column was washed with successive portions of benzene (750 c.c.) until the weights of material removed by consecutive washings were approximately constant. The original filtrate and the washings were collected separately, yielding the fractions I—IX shown in the Table. The column was then divided into three equal parts, each of which was separately eluted with hot chloroform–alcohol. The material obtained from the bottom portion constituted fraction X; that from the remaining two portions has not yet been investigated.

With the exception of fraction I, which was a somewhat viscous yellow oil, all the fractions partly crystallised on standing. Preliminary examination of each fraction showed the presence of β-amyrin in fractions II—VII, and of breïn in fractions VI—X.

Following are the weights (in g.) of the fractions obtained from representative runs, each batch consisting of 150 g. of resin.

Batch No.	Fractions. I.	II.	III.	IV.	V.	VI.	VII.	VIII.	IX.	Total.
1	34·4	73·0	11·18	3·98	2·27	1·54	1·09	0·91	1·33	129·7
2	37·5	64·7	9·65	4·01	2·23	1·40	1·27	1·32	1·58	123·6
3	37·3	66·4	10·87	3·43	2·38	2·09	1·92	1·93	1·75	128·2
4	36·2	66·4	10·40	3·88	2·24	2·00	1·98	1·63	1·75	126·5
5	38·7	70·1	9·62	3·57	2·01	2·21	1·67	1·55	1·79	131·2
6	39·0	64·5	11·37	3·83	2·27	2·04	1·69	1·46	1·62	127·8
7	39·2	70·2	12·53	3·72	2·00	1·63	1·50	1·13	1·27	133·1
8	33·5	66·5	11·75	3·43	2·10	1·72	1·53	1·27	1·28	123·0
9	39·7	68·1	12·98	3·73	2·09	1·63	1·38	1·29	1·38	132·3
10	35·0	66·9	12·27	3·69	2·15	1·65	1·37	1·19	1·30	125·5
Averages	37·05	67·7	11·26	3·73	2·17	1·79	1·54	1·37	1·51	128·1

Removal of β-Amyrin from Fractions VI *and* VII.—The mixed fractions (35 g.) were dissolved in pyridine (35 c.c.) and benzoyl chloride (35 c.c.) and kept at 100° for 2 hours. After standing over-night, the product was decomposed with ice and dilute sulphuric acid, and the mixed benzoates, isolated by means of ether, were taken up in alcohol. The crystalline product (7·5 g.) which separated was digested with boiling methyl alcohol (750 c.c.) for 1 hour, and the insoluble β-amyrin benzoate (6 g.) was filtered off. As no pure substance could be isolated from the filtrate, this was combined with the original mother-liquor, the solvent removed, and the residue heated under reflux for 2 hours with 5% alcoholic potassium hydroxide (40 vols.), the free alcohols being isolated by precipitation with water and extraction with ether.

Isolation of Maniladiol and Breïn.—(A) 28 G. of the mixture of alcohols obtained by the above method were refluxed for 2 hours with benzene (140 c.c.) and formic acid (140 c.c.). Water was then added, and the benzene layer, after addition of ether, was washed with 2% aqueous sodium carbonate and then with water. The residue from the dried and evaporated solution crystallised from aqueous acetone; two recrystallisations yielded fairly pure *maniladiol diformate* (5·4 g.), which after repeated crystallisation (finally from alcohol) formed rosettes of soft needles, m. p. 186—187°, $[\alpha]_D^{17°}$ + 84° (l = 1, c = 2·57) (Found: C, 76·8; H, 10·2. $C_{32}H_{50}O_4$ requires C, 77·0; H, 10·1%). This diformate still contained traces of an impurity, because, although further crystallisation failed to raise the m. p. above 186—187°, formylation of pure maniladiol gave a diformate which had m. p. 191—192° and $[\alpha]_D^{19°}$ + 84° (l = 1, c = 1·35).

Concentration of the mother-liquors of the crude diformate gave 1·2 g. of slightly impure breïn diformate, m. p. 218°. This compound is less soluble than maniladiol diformate, but the latter always crystallises first from mixtures in which it preponderates.

Hydrolysis of maniladiol diformate (m. p. 186—187°) with boiling 2% alcoholic potassium hydroxide, followed by benzoylation of the product and hydrolysis of the benzoate, gave pure *maniladiol*, which separated from aqueous methyl alcohol in rosettes of needles, m. p. 220—221°, $[\alpha]_D^{19°}$ + 68° (l = 1, c = 2·46). The alcohol gave a pink coloration, changing through red to brown, with the Liebermann–Burchard reagent, and a positive tetranitromethane reaction in chloroform (Found: C, 81·0; H, 11·5. $C_{30}H_{50}O_2$ requires C, 81·4; H, 11·4%).

Maniladiol diacetate, prepared by means of pyridine and acetic anhydride, crystallised from methyl alcohol in rosettes of soft needles, m. p. 193—194°, $[\alpha]_D^{20°}$ + 80° (l = 1, c = 1·40) (Found: C, 77·75; H, 10·6. $C_{34}H_{54}O_4$ requires C, 77·5; H, 10·3%).

The *dibenzoate*, which separated in clusters of needles from ethyl alcohol, had m. p. 233—234°, $[\alpha]_D^{17°}$ + 63·5° (l = 1, c = 2·62) (Found : C, 81·0, 80·9; H, 9·2, 9·25. $C_{44}H_{58}O_4$ requires C, 81·2; H, 9·0%).

(B) In earlier experiments the mixture of alcohols (2 parts), freed from β-amyrin, was heated under reflux for 1 hour with acetic anhydride (3 parts) and pyridine (1 part). The mixed acetates, isolated by means of ether after precipitation with water, crystallised readily from alcohol. Fractions VIII, IX, and X were similarly treated, and the crystalline products (m. p. 185—187°) added to that from fractions VI and VII. Continued recrystallisation from alcohol yielded a mixture, m. p. 191—192°, of the diacetates of breïn and maniladiol (average yield, 25% of the mixed alcohols). This m. p. doubtless accounts for the statements, made by earlier authors, that pure breïn diacetate can be obtained by acetylation of impure breïn. Hydrolysis of the mixture, m. p. 191—192°, with 2% alcoholic potash gave the corresponding mixture of diols, m. p. 206—208°, unchanged by crystallisation from alcohol; the use of benzene as solvent led to the isolation of almost pure breïn, m. p. 219—220°, in extremely poor yield. Separation of the diol mixture was readily accomplished by refluxing it with formic acid (25 parts) and benzene (25 parts) for 2 hours. A solution of the formates, isolated as described above, in aqueous acetone deposited breïn diformate, m. p. 220—221° after recrystallisation; maniladiol diformate, m. p. 186—187°, was obtained by concentration of the mother-liquor.

(C) Fractions VIII and IX were combined (30 g.) and converted into the formates exactly as described above. Four recrystallisations of the crude product from aqueous acetone gave *breïn diformate* (3 g.), which separated in large elongated prisms, m. p. 220—221°, $[\alpha]_D^{21°}$ + 67° (l = 1, c = 2·69) (Found : C, 77·0; H, 10·1. $C_{32}H_{50}O_4$ requires C, 77·0; H, 10·1%). The material contained in the first three mother-liquors, after seven recrystallisations from alcohol, yielded maniladiol diformate (0·7 g.).

Hydrolysis of breïn diformate with 2% alcoholic potash gave pure breïn, which separated from aqueous ethyl alcohol in minute prisms, m. p. 221—222°, $[\alpha]_D^{26°}$ + 63·5° (l = 1, c = 2·06), and from aqueous methyl alcohol in needles (Found : C, 81·1; H, 11·4. Calc. for $C_{30}H_{50}O_2$: C, 81·4; H, 11·4%). Vesterberg (*Ber.*, 1906, **39**, 2467) gives m. p. 216—217°, $[\alpha]_D$ + 65·5°; Rollet (*loc. cit.*), m. p. 218—219°; and Mladenović (*loc. cit.*), m. p. 220°. Breïn gives a positive tetranitromethane reaction in chloroform, and a red coloration, changing to brown, with the Liebermann–Burchard reagent.

Breïn diacetate, prepared from the pure diol, formed prisms from aqueous alcohol, m. p. 197—198°, $[\alpha]_D^{17°}$ + 70° (l = 1, c = 1·61) (Found : C, 77·2; H, 10·5. Calc. for $C_{34}H_{54}O_4$: C, 77·5; H, 10·3%). Vesterberg records m. p. 196°, and Rollet, m. p. 195°.

Breïn dibenzoate had m. p. 175—177° after crystallisation from alcohol or aqueous acetone, and m. p. 209—210° when crystallised from ligroin, from which it separated in hard rosettes of needles, $[\alpha]_D^{17°}$ + 58° (l = 1, c = 2·55) (Found : C, 80·8, 81·0; H, 9·4, 9·6. Calc. for $C_{44}H_{58}O_4$: C, 81·2; H, 9·0%). The only data previously recorded for this compound are those of Rollet, who gives m. p. 209—210° for a dried preparation.

Isolation of ψ-Taraxasterol.—Fraction X was freed from elemic acid (approximately 7%) by treatment with 3% alcoholic potassium hydroxide (20 vols.) under reflux for 2 hours. The solution was concentrated to half volume, water added, and the mixture of alcohols isolated by ether-extraction. ψ-Taraxasterol was obtained from the product in each of the following ways :

(a) The mixture of alcohols (60 g.) was formylated by the method already given. A solution of the formates in absolute acetone deposited fairly pure *ψ-taraxasteryl formate* (5·5 g.), which, after recrystallisation from benzene–alcohol, formed needles (4 g.), m. p. 219—221°, $[\alpha]_D^{17°}$ + 51° (l = 1, c = 2·32) (Found : C, 82·1; H, 11·2. $C_{31}H_{50}O_2$ requires C, 81·9; H, 11·1%); the compound is considerably less soluble than the diformates of maniladiol and breïn. The original mother-liquor on concentration yielded a further crop (12 g.) of crystalline material, from which the only isolable compound was a small quantity of breïn diformate.

A sample of ψ-taraxasteryl formate, prepared from authentic ψ-taraxasterol, formed needles from benzene–alcohol, and had m. p. 219—220° both alone and when mixed with the above preparation; $[\alpha]_D^{19°}$ + 51° (l = 1, c = 1·68).

The alcohol obtained by hydrolysis (with boiling 2% alcoholic potash) of ψ-taraxasteryl formate from *Manila elemi* resin separated from alcohol in long needles, m. p. 218—219°, $[\alpha]_D^{17°}$ + 48° (l = 1, c = 2·57) (Found : C, 84·2; H, 11·9. Calc. for $C_{30}H_{50}O$: C, 84·4; H, 11·8%). A mixture of it and an authentic specimen from *Taraxacum* root, m. p. 197—200°, $[\alpha]_D$ + 47°, melted at 200—211° (the m. p. of this specimen was raised to 213—215° by purification *via* the formate; mixed m. p., 213—216°). The alcohol gave a positive tetranitromethane reaction in chloroform, and a magenta-pink coloration in the Liebermann–Burchard test.

The acetate from the *Manila elemi* alcohol crystallised from ethyl alcohol in plates (needles

from benzene–alcohol), m. p. 237—239°, $[\alpha]_D^{17°}$ + 53° (l = 1, c = 2·37) (Found: C, 82·1, 81·9; H, 11·2, 11·1. Calc. for $C_{32}H_{52}O_2$: C, 82·0; H, 11·2%); no depression in m. p. was observed on admixture with authentic ψ-taraxasteryl acetate (m. p. 233—236°, $[\alpha]_D$ + 53°).

The benzoate formed plates from benzene–alcohol, and had m. p. 280—282°, $[\alpha]_D^{17°}$ + 68° (l = 1, c = 2·81) (Found: C, 83·3, 83·2; H, 10·3, 10·2. Calc. for $C_{37}H_{54}O_2$: C, 83·7; H, 10·3%); a mixture with authentic ψ-taraxasteryl benzoate (m. p. 274—276°, $[\alpha]_D$ + 72°) melted at 275—281°. The somewhat low analytical figures for carbon obtained with this compound are possibly due to solvation; a similar result was obtained with the *Taraxacum* specimen (C, 83·3; H, 10·3%).

The melting points of the *Manila elemi* alcohol and its derivatives are in all cases somewhat higher than those of the *Taraxacum* compounds; this is doubtless due to the difficulty experienced in removing from the latter substances the last traces of impurities of similar solubility.

(b) Acetylation of the mixture of alcohols gave a crystalline product (A), m. p. 191—192°, and a non-crystalline residue (B). (A) was separated by hydrolysis and formylation into breïn and maniladiol as already described. Attempts to induce crystallisation of ψ-taraxasteryl acetate from (B) were unsuccessful, but after the material (1 part) had been refluxed for 2 hours with benzene (5 parts) and formic acid (5 parts), ψ-taraxasteryl acetate crystallised readily from a solution of the product in ether–methyl alcohol. Two recrystallisations gave the pure acetate, m. p. 238—240° both alone and when mixed with the sample obtained by method (a).

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