The constituents of the flowers of Anthemis nobilis / by Frederick Belding Power and Henry Browning, Jun.

CLXXII.—*The Constituents of the Flowers of* Anthemis nobilis.

By Frederick Belding Power and Henry Browning, jun.

The flower-heads of the composite plant, *Anthemis nobilis*, Linné, commonly known as chamomile flowers, or, more specifically, as the Roman or English chamomile, are used to a considerable extent medicinally, and are recognised by the British, United States, and other national Pharmacopœias.

Although the above-mentioned flowers have hitherto been the subject of several investigations, apart from the essential oil which is yielded by their distillation with steam, and a substance designated as anthesterol, comparatively little of a definite nature has been known respecting their constituents.

Camboulises (*J. Pharm. Chim.*, 1871, [iv], **14**, 337) has stated that the flowers contain, besides wax, fat, and dextrose, an acid resembling the so-called "anthemic acid" of Pattone (*ibid.*, 1859, [iii], **35**, 198), but which, as in the case of the latter product, was not definitely characterised, and was doubtless an impure substance. Naudin (*Bull. Soc. chim.*, 1884, [ii], **41**, 483) has recorded the isolation of a hydrocarbon, $C_{18}H_{36}$ (m. p. 63—64°), termed anthemene, and a crystalline substance (m. p. 188—189°) termed anthemol, whilst Klobb, Garnier, and Ehrwein (*ibid.*, 1910, [iv], **7**, 948) state that they found a hydrocarbon of the composition $C_{30}H_{62}$.

Klobb (*Bull. Soc. chim.*, 1902, [iii], **27**, 1229) obtained from the flower-heads of the Roman chamomile a crystalline substance, designated "anthesterin" (anthesterol), which was regarded as a new compound belonging to the class of phytosterols. It was stated to melt at 221—223°, to have $[\alpha]_D$ +48·3° (in ethylene bromide), and to possess the formula $C_{28}H_{48}O$ or $C_{29}H_{50}O$. The same investigator (*Ann. Chim. Phys.*, 1909, [viii], **18**, 135) subsequently described α- and β-modifications of the benzoyl derivative of anthesterol, and stated that under certain conditions, which were difficult to determine, the β-compound is converted into a γ-modification (compare also *Compt. rend.*, 1909, **148**, 1272). In a later publication (*Compt. rend.*, 1911, **152**, 327) anthesterol was considered to possess the formula $C_{31}H_{52}O,3H_2O$, to melt at 195°, and, in the anhydrous state, to have $[\alpha]_D$ +79·4° (in chloroform). It was, furthermore, stated to have been resolved, by means of its acetyl derivatives, into three isomerides of different melting points, and, although the acetyl derivatives yielded bromo-compounds of varying composition, the anthesterol was, nevertheless, considered

to be a homogeneous substance. The most recent communication on the subject by the same author (*Ann. Chim. Phys.*, 1911, [viii], **24**, 134) includes a statement that anhydrous anthesterol has $[\alpha]_D$ +75°4′. Cohen (*Arch. Pharm.*, 1908, **246**, 520) has considered it probable that the anthesterol of Klobb is identical with lupeol, to which the doubtful formula $C_{26}H_{42}O$ has been assigned. It would appear, however, from the discrepant and confusing statements above noted that the "anthesterol" of Klobb must have consisted of a mixture of substances, and confirmation of this view has been afforded by the results of the present investigation.

Flückiger and Hanbury ("Pharmacographia," 1879, p. 386) could only obtain the bitter principle of the Roman chamomile in the form of a brown extract, which they state is apparently a glucoside, and the absence of an alkaloid was confirmed by them.

In view of the existing deficiency of knowledge, it was deemed desirable to submit chamomile flowers to a more complete examination, and the results of the present research are summarised at the end of this paper.

Experimental.

The material employed for this investigation consisted of the flower-heads of *Anthemis nobilis*, Linné, collected from plants grown in Belgium.

Fifteen grams of the ground material were digested with Prollius' fluid, and the resulting extract tested with the usual alkaloid reagents. The reactions thus obtained indicated the presence of an appreciable amount of an organic base.

An examination of the flowers for the presence of an enzyme soluble in water gave a negative result.

Twenty-five grams of the ground flowers were successively extracted in a Soxhlet apparatus with various solvents, when the following amounts of extract, dried in a water-oven, were obtained:

Petroleum (b. p. 35—50°) extracted	1·07 grams	=	4·28	per cent.
Ether ,,	3·07 ,,	=	12·28	,,
Chloroform ,,	0·08 ,,	=	0·32	,,
Ethyl acetate ,,	3·00 ,,	=	12·00	,,
Alcohol ,,	2·08 ,,	=	8·32	,,
Total	9·30 grams	=	37·20	per cent.

For the purpose of a complete examination, 21·09 kilograms of the ground material were thoroughly extracted by percolation with hot alcohol. After the removal of the greater part of the alcohol, 8·33 kilograms of a viscid, brownish-green extract were obtained.

The whole of this extract was mixed with water and subjected to distillation in a current of steam, when 34·3 grams of a pale yellow essential oil were obtained, which distilled for the most part between 170° and 210° under ordinary pressure. As this product did not represent a normal oil, and as the latter, obtained by the direct distillation of the flowers with steam, has previously been chemically examined, it was not deemed of further interest.

Non-volatile Constituents of the Extract.

After the distillation of the extract with steam there remained in the distillation flask a dark brown aqueous liquid (*A*), which contained a quantity of suspended resin, together with fatty material which floated on the surface. The aqueous liquid was decanted and filtered, the resin being thus collected, and then thoroughly washed with water, whilst the fatty material was separately treated in a similar manner. The washings having been added to the main portion of the aqueous liquid, the fat and resin were subsequently brought together, and represent the product described as the resin (*B*).

Examination of the Aqueous Liquid (A).

The filtered aqueous liquid, on keeping, deposited a small amount of a nearly colourless substance, mixed with some resinous material. This substance was purified by repeated separation from dilute alcohol, when it appeared to be crystalline, and melted at 175—178°, although the liquid did not become clear until a temperature of about 230° was reached. It yielded an acetyl derivative melting at 144°, and evidently consisted of the glucoside of apigenin, which was subsequently isolated in larger amount, and is fully described below.

After the removal of the small amount of glucosidic substance the aqueous liquid was concentrated under diminished pressure, and a portion of the liquid which represented 4 kilograms of the original extract was shaken with twelve successive portions of ether. The ethereal extracts were united, and, by the evaporation of a small aliquot portion, were found to contain about 70 grams of solid material, which possessed an intensely bitter taste, but no alkaloid was present. The total ethereal extract, after concentration, was shaken successively with aqueous ammonium carbonate, sodium carbonate, and sodium hydroxide.

Isolation of 3:4-Dihydroxycinnamic Acid.

The ammonium carbonate extract was acidified and extracted with ether, when a tar-like product was obtained. This was esterified, and the ethereal solution of the ester extracted with a 1 per cent. solution of potassium hydroxide. From the non-phenolic portion of the ester nothing definite could be isolated, but from the phenolic portion, after hydrolysis, 0·35 gram of an acid was obtained, which separated from water containing a trace of alcohol in small, yellow needles, melting at 220—223°. After drying at 120°, the substance was analysed, and proved to be 3:4-dihydroxycinnamic acid. (Found, C = 60·1; H = 4·5. Calc., C = 60·0; H = 4·4 per cent.)

The sodium carbonate extract of the ethereal liquid yielded, on acidification, 0·7 gram of brown material, which was separated several times from dilute alcohol, but could not be obtained in a definitely crystalline state. It melted and decomposed at 334—336°, and yielded a colourless acetyl derivative, melting at 176—177°. The analysis of the substance and of its acetyl derivative proved it to be apigenin, $C_{15}H_{10}O_5$, which was subsequently obtained by the hydrolysis of its glucoside, and was also found to be contained in considerable amount, in the free state, in the ethereal extract of the resin, as noted below.

The sodium hydroxide extract of the original ethereal liquid yielded, on acidification, 0·7 gram of a substance which was fairly soluble in water, gave no definite coloration with ferric chloride, and, when crystallised from ether, separated in colourless needles, melting at 143°. An analysis of the substance gave C = 64·0; H = 7·4 per cent., but the amount was too small to permit of its further characterisation. The ethereal liquid, after its successive extraction with alkalis, was evaporated, but no pure substance could be isolated from the residual neutral material.

One-half of the original aqueous liquid which had been thoroughly extracted with ether was next shaken repeatedly with warm amyl alcohol. This removed about 193 grams of material, which possessed an intensely bitter taste. The combined amyl alcohol extracts were concentrated, when after some time a quantity of solid material separated. This was collected, washed with light petroleum, and then mixed with a little water and heated in a current of steam, in order to remove the amyl alcohol as completely as possible. This process of concentrating the amyl alcohol extract was repeated three times, and the material which separated was treated in each case as above described. To the small propor-

tion of amyl alcohol extract remaining after the preceding separations ether was first added and subsequently chloroform, the resulting precipitates being separately collected, and heated in a current of steam as before for the removal of adhering amyl alcohol. The products which had been treated with steam were separated from the aqueous liquid by filtration, and were thus obtained in the form of brown, viscid masses.

Isolation of an Apigenin-d-glucoside, $C_{21}H_{20}O_{10},H_2O$.

As all the above-mentioned portions of brown, viscid material were similar in character they were mixed, and then separated several times from large volumes of very dilute alcohol. It was thus obtained in a form which permitted of its being dried, but if smaller volumes of solvent were used the material was deposited as an oil. After further successive separations from 30 per cent. acetic acid, and alcohol of about 20 per cent. strength, the material was finally obtained in microscopic crystals having a slightly yellow tint. This crystalline substance melted and decomposed at 178—180°, and was found to be a glucoside. Considerable difficulty was experienced in drying the substance, on account of its great avidity for moisture. It was practically devoid of bitterness, and possessed only a slightly astringent taste. Its aqueous solution gave with alkalis a lemon-yellow colour, and with ferric chloride a purplish-brown coloration was produced:

0·1321, heated at 125—130° for several days, lost 0·0106 H_2O. $H_2O=8·0$.

0·3528, dried in a desiccator over calcium chloride, lost 0·0282 H_2O. $H_2O=8·0$.

0·1215 * gave 0·2487 CO_2 and 0·0543 H_2O. $C=55·8$; $H=5·0$.

0·1820 † „ 0·3733 CO_2 „ 0·0826 H_2O. $C=55·9$; $H=5·0$.

$C_{21}H_{22}O_{11}$ requires $C=56·0$; $H=4·9$ per cent.

$C_{21}H_{22}O_{11},2H_2O$ requires $H_2O=7·4$ „ „

It will be seen from these results that the above-described substance, when subjected to prolonged drying at 125—130°, or when dried over calcium chloride, agrees in its empirical composition with the formula $C_{21}H_{22}O_{11}$, whilst the loss of water under the conditions mentioned is equivalent approximately to two molecules. No further amount of water could be removed from the substance, since at a temperature above 130° it showed a tendency to soften and decompose.

A consideration of the composition of the acetyl derivative and hydrolytic products of the substance rendered it evident that it

* Dried at 125—130°. † Dried over calcium chloride.

was a glucoside of apigenin (1:3:4′-trihydroxyflavone), $C_{15}H_{10}O_5$, but that, in addition to water of crystallisation, it contained a molecule of water which could not be eliminated without decomposition. The formula of the glucoside is therefore to be represented as $C_{21}H_{20}O_{10},H_2O$.

A compound obtained by Vongerichten (*Annalen*, 1901, **318**, 124; 1902, **321**, 71) by the partial hydrolysis of apiin, and designated as *d*-glucoseapigenin, was stated to melt at 215—220°, and, when dried at 120—130°, to possess the formula $C_{21}H_{20}O_{10}$. When crystallised from dilute alcohol it contained an amount of water agreeing approximately with 2 molecules. Inasmuch as the apigenin glucoside obtained from chamomile flowers differed in some respects from the compound described by Vongerichten (*loc. cit.*), especially in its melting point and the elements of an additional molecule of water, it was deemed desirable to prepare some of the latter compound for the purpose of comparison. The product obtained by the partial hydrolysis of a commercial specimen of apiin, in exact accordance with the method given by Vongerichten (*loc. cit.*), was found to melt at 192—195°, the higher melting point (215—220°) recorded by the last-mentioned investigator having probably been due to the presence of a little apigenin produced by further hydrolysis, which was also indicated by the results of his analysis of *d*-glucoseapigenin. It would appear that the latter compound undergoes partial hydrolysis on simply boiling its aqueous solutions, and in this respect also it differs from the glucoside obtained from chamomile flowers, which remains quite unchanged by this treatment. The product from apiin, melting at 192—195°, was analysed with the following result:

0·1084, on heating at 110—120°, lost 0·0092 H_2O. $H_2O = 8·5$.
0·0992 * gave 0·2120 CO_2 and 0·0440 H_2O. $C = 58·3$; $H = 4·9$.
$C_{21}H_{20}O_{10}$ requires $C = 58·3$; $H = 4·6$ per cent.
$C_{21}H_{20}O_{10},2H_2O$ requires $H_2O = 7·5$ „ „

With consideration of these results, the *apigenin*-d-*glucoside* obtained from chamomile flowers is to be regarded as a new compound.

Hexa-acetylapigenin-d-*glucoside*, $C_{21}H_{14}O_{10}(CO·CH_3)_6$.—A portion of the glucoside was heated for some time with acetic anhydride containing a small proportion of pyridine. The product of the reaction, after removing the greater part of the acetic anhydride, was poured into water, and the deposited substance collected. This substance, when separated from a mixture of alcohol and ethyl acetate, was obtained in colourless, microscopic crystals, melting

* Dried at 110—120°.

at 144—146°. It contained water of crystallisation, which was only completely eliminated with difficulty:

0·1057, on heating at 110°, lost 0·0102 H_2O. $H_2O = 9·6$.
0·0955 * gave 0·2013 CO_2 and 0·0419 H_2O. $C = 57·5$; $H = 4·9$.
$C_{33}H_{32}O_{16}$ requires $C = 57·9$; $H = 4·7$ per cent.
$C_{33}H_{32}O_{16},4H_2O$ requires $H_2O = 9·5$ „ „

It will be seen from the above results that the molecule of water which could not be removed from the glucoside without decomposition was eliminated in the process of acetylation.

*Hydrolysis of Apigenin-*d*-glucoside.*

Formation of Apigenin, $C_{15}H_{10}O_5$, *and Dextrose.*

A portion of the glucoside was boiled with 5 per cent. aqueous sulphuric acid for about three hours, when a yellow product separated and was collected. This substance was insoluble in water, but fairly soluble in hot alcohol, and readily so in pyridine, from which on dilution it separated in yellow, microscopic needles, melting at 345—350°. Its alcoholic solution gave with ferric chloride a brown coloration, and with alkalis a deep yellow colour was produced. It dissolved in aqueous sodium carbonate, forming a bright yellow solution, and, on acidification, was again precipitated in a yellow state. A much larger amount of the same substance was subsequently obtained from the ethereal extract of the resin. After drying at 110—115°, it was analysed. (Found, $C = 66·2$; $H = 3·9$; $C = 66·4$; $H = 4·1$. $C_{15}H_{10}O_5$ requires $C = 66·7$; $H = 3·7$ per cent.)

The properties and analysis of the above-described substance render it evident that it is apigenin, and its identity was further confirmed by the preparation of the derivatives described below.

For the purpose of comparison, an attempt was made to determine, by the microscopical method in pyridine solution, the molecular weight of the apigenin from chamomile flowers and that prepared by the hydrolysis of apiin, but in both instances abnormally low results were obtained.

Triacetylapigenin, $C_{15}H_7O_5(CO·CH_3)_3$.—This derivative was prepared by heating apigenin with acetic anhydride containing a small proportion of pyridine. When crystallised from methyl alcohol, it separated in colourless, silky needles, melting at 176—177° (compare Czajkowski, v. Kostanecki, and Tambor, *Ber.*, 1900, **33**, 1993). After drying at 110°, an analysis and a determination of the molecular weight (by the cryoscopic method in benzene) were

* Dried at 110°.

made. (Found, C = 63·6; H = 4·2. M.W. = 382. $C_{21}H_{16}O_8$ requires C = 63·6; H = 4·2 per cent. M.W. = 396.)

A solution of 0·2346 gram of the acetyl derivative in 20 c.c. of chloroform had no discernible optical rotation.

Apigenin 3:4′-Dimethyl Ether, $C_{15}H_7O_2(OMe)_2 \cdot OH$.—This derivative was prepared with the use of methyl sulphate and an excess of potassium hydroxide. When crystallised from a mixture of alcohol and ethyl acetate it separated in pale yellow needles, melting at 168°. It was dried at 110°, and analysed. (Found, C = 67·9; H = 5·0; OMe = 19·3. Calc., C = 68·4; H = 4·7; OMe = 20·8 per cent.)

*Examination of the Sugar yielded by the Hydrolysis of Apigenin-*d*-glucoside.*

The aqueous liquid resulting from the hydrolysis of the above-described glucoside was treated with baryta for the removal of the sulphuric acid, and, after filtration, was concentrated. It then readily yielded an osazone, which melted and decomposed at 212°, and was evidently *d*-phenylglucosazone.

The above results have thus shown the glucoside to be resolved on hydrolysis into apigenin and dextrose. A known quantity of the glucoside, when heated with 5 per cent. sulphuric acid, yielded apigenin equal to 58·3 per cent. of its weight ($C_{21}H_{20}O_{10},H_2O$ requires $C_{15}H_{10}O_5 = 60$ per cent.). The hydrolysis of the apigenin-*d*-glucoside therefore takes place according to the following equation:

$$C_{21}H_{20}O_{10},H_2O = C_{15}H_{10}O_5 + C_6H_{12}O_6.$$

The original aqueous liquid (*A*), which had been extracted with ether and amyl alcohol as already described, was next treated with a slight excess of basic lead acetate. A large amount of a pale brown precipitate was thus produced, which was collected and well washed with water. A portion of this precipitate was suspended in water and decomposed by hydrogen sulphide, the mixture being then filtered, and the filtrate concentrated under diminished pressure. The liquid thus obtained gave a greenish-black coloration with ferric chloride, but no precipitate with gelatin, and therefore appeared to contain no tannin. Separate portions of the liquid were subsequently so treated as to contain 5 per cent. of sulphuric acid and 10 per cent. of potassium hydroxide respectively, and the solutions then boiled for some time, but they yielded nothing definite.

The filtrate from the precipitate produced by basic lead acetate was treated with hydrogen sulphide for the removal of the excess

of lead, and the filtered liquid concentrated under diminished pressure to the consistency of a syrup. It was found to contain a quantity of sugar, since it readily yielded *d*-phenylglucosazone, melting at 208—210°. A small portion of the syrupy liquid was tested with mercuric nitrate, but no base precipitable by this reagent was present.

Isolation of Choline, $C_5H_{15}O_2N$.

The chief portion of the above-mentioned syrupy liquid was extracted with alcohol, the alcoholic liquid evaporated, and the residue again treated with alcohol, this operation being repeated until a product was finally obtained which was soluble in nearly absolute alcohol. Subsequently the remaining portion of the original aqueous liquid (*A*) which had been extracted with ether, together with the remainder of the aqueous liquid from the original alcoholic extract of the chamomile flowers, was extracted with amyl alcohol, then purified by treatment with basic lead acetate, concentrated, and treated with alcohol as above described. To the total amount of alcoholic liquid which had thus been obtained a saturated alcoholic solution of mercuric chloride was added, and the mixture kept for several days. The precipitate which had then formed was collected, washed with a little alcohol, dissolved as completely as possible in warm water, and the mercury removed from the solution by means of hydrogen sulphide. To the filtered aqueous liquid, slightly acidified with sulphuric acid, a solution of phosphotungstic acid was added, the precipitate thus produced being treated with barium hydroxide, the mixture filtered, and the excess of barium removed by carbon dioxide. The clear solution was then neutralised with hydrochloric acid and evaporated, first under diminished pressure and finally in a vacuum desiccator, to dryness. The residue was repeatedly extracted with absolute alcohol until a product was obtained which was completely soluble in that liquid, and, by the evaporation of the solvent, formed very deliquescent, needle-shaped crystals, which possessed the characters of choline chloride. The portion of the residue which remained undissolved by treatment with absolute alcohol gave no indication of the presence of betaine.

A portion of the above-mentioned crystalline substance, when dissolved in water, gave with a solution of auric chloride a pale yellow precipitate. This was collected, washed with a little water, and dried at 105°. (Found, Au = 44·0. $C_5H_{14}ONCl,AuCl_3$ requires Au = 44·5 per cent.)

Another portion of the crystalline substance was converted into the platinic chloride double salt, which crystallised from water in

orange-coloured needles, melting and decomposing at 258—262°. (Found, C=19·4; H=4·9; Pt=31·7. $(C_5H_{14}ONCl)_2PtCl_4$ requires C=19·5; H=4·5; Pt=31·7 per cent.)

The amount of choline, $C_5H_{15}O_2N$, obtained in the form of the pure gold and platinum salts was equivalent to about 0·005 per cent. of the weight of chamomile flowers employed, but, with consideration of its incomplete separation from the extract, it must have been present in a considerably larger proportion.

Isolation of i-*Inositol*, $C_6H_6(OH)_6$.

A small portion of the same syrupy liquid as had been employed for the isolation of choline was deprived as completely as possible of water, and then heated with acetic anhydride in the presence of a trace of *d*-camphorsulphonic acid. A quantity of the syrupy deposit remaining after the extraction of the choline by alcohol was likewise treated in the same manner. After purification of the acetylated products, a substance was obtained which separated from 70 per cent. alcohol in colourless, glistening plates. A portion (0·5 gram) of this substance was hydrolysed by heating with 5 per cent. sulphuric acid in a current of steam, and the product crystallised from dilute alcohol containing a trace of ether. Colourless needles were thus obtained, which melted at 220—224°, and when mixed with a known specimen of inositol no depression of the melting point ensued. The compound melting at 220—224° was dried at 120° and analysed. (Found, C=40·1; H=6·9. Calc., C=40·0; H=6·7 per cent.)

The above-described acetyl derivative melted at 205—207°, and was devoid of optical activity. After drying at 110°, it was analysed. (Found, C=49·6; H=5·6. $C_6H_6O_6(CO\cdot CH_3)_6$ requires C=50·0; H=5·6 per cent.)

The presence of *i*-inositol in chamomile flowers was thus definitely established.

Examination of the Resin (B).

The product consisting of resinous and fatty material, which had been separated from the aqueous liquid (*A*), as previously described, amounted to about 7·4 per cent. of the weight of chamomile flowers employed.

Inasmuch as a preliminary test of the chamomile flowers had shown the presence of an appreciable amount of an organic base, it was deemed desirable to ascertain whether such a substance was contained in the resinous material. A portion of this material, representing 4·33 kilograms of the original alcoholic extract, was therefore repeatedly extracted with 5 per cent. sulphuric acid.

The combined aqueous liquids were treated with baryta for the removal of the sulphuric acid, and the slight excess of baryta subsequently removed by means of carbon dioxide, after which the liquid was concentrated to a small volume and slightly acidified with hydrochloric acid. A small amount of dark-coloured, amorphous material was thus precipitated, which contained nothing definite. By then treating the aqueous liquid with phosphotungstic acid, decomposing the precipitate so produced with baryta, and finally precipitating the product with an alcoholic solution of mercuric chloride, essentially as described above in connexion with the isolation of choline, a further small amount of this substance was obtained. It yielded a platinum salt, which was analysed. (Found, Pt=31·4. $(C_5H_{14}ONCl)_2PtCl_4$ requires Pt=31·7 per cent.)

As the resinous and fatty material from which this small amount of choline was obtained had previously been thoroughly washed with hot water, it seems probable that the base was partly contained in the chamomile flowers in the form of a compound insoluble in water.

The remainder of the resinous material, representing 4 kilograms of the original alcoholic extract, was mixed with purified sawdust, the mixture thoroughly dried, and then successively extracted in a large Soxhlet apparatus with various solvents. The amounts of the extracts thus obtained, when dried in a steam-oven, were as follows:

Petroleum (b. p. 35—50°) extracted	369	grams.
Ether	138	,,
Chloroform	38	,,
Ethyl acetate	85	,,
Alcohol	117	,,
Total	747	grams.

Petroleum Extract of the Resin.

During the extraction of the resinous material with light petroleum, 55 grams of crystalline substance separated, which had a slight green colour, due to chlorophyll. A further amount of similar substance was obtained after the alkaline hydrolysis of the extract, and the examination of this material is described below. After the separation of the above-mentioned substance from the light petroleum, the solvent was removed, and the residue heated with an alcoholic solution of potassium hydroxide. The alcohol was then evaporated, water added, and the alkaline mixture extracted with ether.

Isolation of Triacontane, $C_{30}H_{62}$.

By the above-mentioned extraction of the aqueous alkaline liquid with ether a quantity of material was obtained, which was first crystallised several times from alcohol. A sparingly soluble fraction of low melting point was thus obtained, together with a number of products of higher melting points. The more sparingly soluble substance was crystallised several times from ethyl acetate, when it separated in colourless, glistening leaflets, melting at 64—65°, and amounted to 4·5 grams. This substance was identified as triacontane (Found, C = 84·9; H = 14·7. Calc., C = 85·3; H = 14·7 per cent.). A hydrocarbon agreeing in composition with the formula $C_{30}H_{62}$ (m. p. 64°) had previously been isolated by Klobb, Garnier, and Ehrwein (*Bull. Soc. chim.*, 1910, [iv], **7**, 948) from the flowers of *Anthemis nobilis*.

Isolation of Taraxasterol, $C_{29}H_{47}\cdot OH$.

The portion of crystalline substance which had separated from the original petroleum extract was fractionally crystallised from alcohol, and some of these fractions were incorporated with those of similar melting points which had been obtained in the course of the above-described separation of triacontane. All the portions of crystalline material were subsequently subjected to prolonged fractional crystallisation, first from alcohol, and afterwards from ethyl acetate. The fractions were then separately acetylated, and the process of fractional crystallisation continued with the use of ethyl acetate. A quantity (about 9·5 grams) of a substance was thus finally obtained in colourless, lustrous plates, which melted constantly at 248—250°. As this substance possessed practically the same melting point and characters as the acetyl derivative of the monohydric alcohol taraxasterol, previously isolated by the present authors from taraxacum root (T., 1912, **101**, 2423), a little of it was mixed with the last-mentioned acetyl derivative, when no depression of the melting point ensued. A portion of the above-described acetylated substance (m. p. 248—250°) was hydrolysed, and the resulting compound crystallised from alcohol, when it separated in small, colourless needles, melting at 217—219°. When mixed with taraxasterol no change in melting point was observed. (Found, $H_2O = 9·3$. $C_{29}H_{48}O,2\frac{1}{2}H_2O$ requires $H_2O = 9·8$ per cent. After being dried at 120°, Found, C = 84·4; H = 11·7. $C_{29}H_{48}O$ requires C = 84·5; H = 11·6 per cent.)

The optical rotatory power of the substance was determined with the following result:

0·2855,* made up to 25 c.c. with chloroform, gave α_D +2°11′ in a 2-dcm. tube, whence $[\alpha]_D$ +95·6°.

When a small amount of the substance is dissolved in chloroform with a little acetic anhydride, and a few drops of concentrated sulphuric acid subsequently added, a pink colour is produced, which slowly changes to a dark magenta with a green fluorescence.

The composition and characters of the above-described substance rendered it evident that it was taraxasterol, and its identity with the latter was further confirmed by an examination of the acetyl derivative. (Found, C=81·7; H=11·2. $C_{31}H_{50}O_2$ requires C=81·9; H=11·0 per cent.)

A determination of its optical rotatory power gave the following result:

0·3666,* made up to 20 c.c. with chloroform, gave α_D +3°37′ in a 2-dcm. tube, whence $[\alpha]_D$ +98·7°.

The specific rotation of acetyltaraxasterol was previously recorded (*loc. cit.*, p. 2424) as +122·2°, but this evidently involved an error, since a redetermination of the rotation of the same specimen of the substance has given $[\alpha]_D$ +102·5°.

The monobromoacetyl derivative (m. p. 235—237°) was also prepared. (Found, C=69·9; H=9·4. $C_{31}H_{49}O_2Br$ requires C=69·8; H=9·2 per cent.)

In the process of separating the above-described acetyltaraxasterol several fractions of lower melting point were obtained, and two of these were more completely examined, with the following results:

I. M. p. 235—237°; $[\alpha]_D$ +91·1°; C=81·7; H=11·1 per cent.
II. M. p. 220—225°; $[\alpha]_D$ +69·5°; C=81·3; H=11·2 „ „

The composition and character of these fractions indicate that taraxasterol is accompanied in the chamomile flowers by one or more analogous substances, possessing both a lower melting point and a lower optical rotation.

Inasmuch as it is now known that phytosterols of the composition $C_{27}H_{46}O$ frequently occur in plants in the form of their glucosides, and also that such glucosides can be prepared synthetically (T., 1913, **103**, 399, 1022), it was deemed of interest to ascertain whether taraxasterol is capable of forming such a compound. A quantity of anhydrous taraxasterol and pure bromoacetoglucose, dissolved in dry ether, were accordingly shaken for several hours with freshly precipitated dry silver oxide, but no trace of glucosidic substance could be found in the product.

* Dried at 120°.

Examination of the Fatty Acids.

The previously mentioned aqueous, alkaline liquid, which had been extracted with ether for the removal of the unsaponifiable material, was next acidified, and again extracted with ether. The fatty acids thus obtained were converted into their methyl esters, the latter amounting to 65 grams. These esters were distilled twice under diminished pressure, when they passed over between 200° and 275°/25 mm., and were found to be optically inactive. The esters were subsequently hydrolysed, and the acids separated into liquid and solid portions by conversion into their lead salts and treatment of the latter with ether.

The Liquid Acids.—These acids, when distilled twice under diminished pressure, passed over between 200° and 230°/10 mm., and amounted to 4 grams. They gave on analysis C=76·9; H=11·5, and had an iodine value of 153·3:

$C_{18}H_{34}O_2$ requires C=76·6; H=12·1 per cent. Iodine value=90·1.
$C_{18}H_{32}O_2$ „ C=77·1; H=11·4 „ „ Iodine value=181·4.

The liquid acids thus appear to consist of a mixture of oleic and linolic acids, the latter predominating.

The Solid Acids.—These acids, amounting to 14 grams, were fractionally crystallised from a mixture of alcohol and ethyl acetate, when a small fraction was obtained, which melted at 76—77°, and evidently consisted of cerotic acid. (Found, C=78·5; H=13·2. $C_{27}H_{54}O_2$ requires C=79·0; H=13·2 per cent.)

The main fraction of acid melted at 52—54°. It gave on analysis C=75·4; H=12·7, and had a neutralisation value of 200:

$C_{16}H_{32}O_2$ requires C=75·0; H=12·5 per cent. N.V.=219·1.
$C_{18}H_{36}O_2$ „ C=76·1; H=12·7 „ „ N.V.=197·5.

This fraction of acid thus appeared to be a mixture of palmitic and stearic acids.

Ether Extract of the Resin.

Isolation of a Phytosterol Glucoside.

The ether extract of the resin was kept for some time before the removal of the solvent, when it deposited 11 grams of material, which had a green colour, due to chlorophyll. This material was collected and purified by treatment with dilute pyridine, from which it separated in nearly colourless, microscopic crystals, melting at 280—283°. It was dried at 120° and analysed:

0·1031 gave 0·2757 CO_2 and 0·0982 H_2O. C=72·9; H=10·6.
$C_{33}H_{56}O_6$ requires C=72·3; H=10·2 per cent.
$C_{36}H_{60}O_6$ „ C=73·5; H=10·2 „ „

A portion of the substance was acetylated, and the product crystallised from alcohol, when it separated in small, needle-shaped crystals, melting at 159—160°. After drying at 110°, it was analysed:

0·0958 gave 0·2421 CO_2 and 0·0787 H_2O. C = 68·9; H = 9·1.

$C_{33}H_{52}O_6(CO{\cdot}CH_3)_4$ requires C = 68·7; H = 8·9 per cent.

$C_{36}H_{56}O_6(CO{\cdot}CH_3)_4$ „ C = 69·8; H = 9·0 „ „

The above results would thus indicate that the substance melting at 280—283° was a mixture of the glucosides of sitosterol, $C_{27}H_{46}O$, and stigmasterol, $C_{30}H_{50}O$. Its identity as a phytosterolin was established by hydrolysing it in amyl alcohol solution by means of hydrochloric acid. The phytosterol so produced was fractionally crystallised several times from a mixture of alcohol and ethyl acetate, when the main portion was obtained in glistening plates, melting at 136—139°:

0·1991, on heating at 115°, lost 0·0089 H_2O. H_2O = 4·5.

0·0993 * gave 0·3044 CO_2 and 0·1060 H_2O. C = 83·6; H = 11·9.

$C_{27}H_{46}O$ requires C = 83·9; H = 11·9 per cent.

$C_{27}H_{46}O,H_2O$ requires H_2O = 4·4 per cent.

The original phytosterolin thus appeared to consist, for the most part, of a sitosterol glucoside.

The ethereal liquid from which the crude phytosterolin had been removed was shaken several times with an aqueous 8 per cent. solution of hydrogen chloride, when a considerable amount of an emulsion was formed. The acid liquids gave reactions with the usual alkaloid reagents, but, when made alkaline and extracted with both ether and chloroform, nothing definite could be obtained from them. The material which separated from the above-mentioned emulsion was washed with ether, and a portion of it heated in aqueous alcohol with dilute hydrochloric acid. A small amount of apigenin was thus obtained, but the material appeared to remain for the most part unchanged. This unchanged material, together with the remainder of the original portion, was then heated for about ten minutes with a 50 per cent. aqueous solution of potassium hydroxide, the product poured into a slight excess of cold dilute hydrochloric acid, and the mixture extracted with ether. On shaking this ethereal liquid with aqueous ammonium carbonate, a very small amount of an acid was obtained, which, when crystallised from water, melted at 207—209°, and gave a dark yellow coloration with ferric chloride. The substance was evidently *p*-hydroxybenzoic acid, which, together with *p*-hydroxyacetophenone, is known to be formed by the alkaline hydrolysis of apigenin

* Anhydrous substance

(T., 1897, **71**, 810). After shaking the ethereal liquid with ammonium carbonate, it was extracted with aqueous potassium hydroxide, which removed some dark-coloured material. The latter, when treated with petroleum of high boiling point, yielded a substance which crystallised from benzene in small, nearly colourless needles, melting at 105—107°, and was identified as *p*-hydroxyacetophenone (Found, C=70·5; H=5·9. Calc., C=70·6; H=5·9 per cent.). A small amount of the substance was converted into the oxime, which melted at 139—140°.

The original ethereal extract of the resin, which had been shaken with aqueous hydrogen chloride, as above described, was next washed with water, and then shaken successively with aqueous ammonium carbonate, sodium carbonate, and sodium hydroxide, the ether being finally evaporated. All of these products were of a resinous nature, with the exception of the material removed by sodium carbonate, from which ultimately 9 grams of pure apigenin were obtained.

Chloroform, Ethyl Acetate, and Alcohol Extracts of the Resin.

These extracts were separately examined, but they yielded nothing of further interest. The ethyl acetate extract evidently contained some of the previously described glucoside of apigenin, for, after heating with dilute hydrochloric acid, a small amount of apigenin was obtained, together with a sugar which yielded an osazone melting at 212—215°.

Summary.

The material employed for this investigation consisted of the flower-heads of *Anthemis nobilis*, Linné, collected from plants grown in Belgium.

Apart from the essential oil yielded by distillation with steam, the flowers have been found to contain the following definite compounds: (i) 3:4-dihydroxycinnamic acid; (ii) apigenin, $C_{15}H_{10}O_5$; (iii) a *glucoside* of *apigenin*, $C_{21}H_{20}O_{10},H_2O$ (m. p. 178—180°), which yields an *hexa-acetyl* derivative, $C_{33}H_{32}O_{16},4H_2O$, melting at 144—146°; (iv) choline, $C_5H_{15}O_2N$; (v) *i*-inositol, $C_6H_6(OH)_6$; (vi) triacontane, $C_{30}H_{62}$; (vii) taraxasterol, $C_{29}H_{47}\cdot OH$ (m. p. 217—219°); (viii) a phytosterolin (m. p. 280—283°), consisting chiefly of sitosterol-*d*-glucoside, $C_{33}H_{56}O_6$; (ix) a mixture of fatty acids, consisting of cerotic, stearic, palmitic, oleic, and linolic acids. The flowers contained, furthermore, a considerable quantity of sugar, which yielded *d*-phenylglucosazone (m. p. 208—210°). The amount of fatty and resinous material, from which some of the

above-mentioned substances were obtained, was equivalent to about 7·4 per cent. of the weight of flowers employed.

The so-called "anthemic acid" of previous investigators was evidently a very indefinite product, while the "anthesterol" of Klobb was doubtless a mixture, consisting chiefly of the compound which has been designated by the authors as taraxasterol.

The bitter taste of chamomile flowers appears to be due to dark-coloured, amorphous material, and not to any well-defined constituent. It was found, for example, that the portion of the alcoholic extract which is soluble in water, when extracted successively with ether and amyl alcohol, yielded viscous products, which possessed an intensely bitter taste.

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