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THE IDENTIFICATION OF IPURANOL AND SOME ALLIED COMPOUNDS AS PHYTOSTEROL GLUCOSIDES

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XLVII.—*The Identification of Ipuranol and Some Allied Compounds as Phytosterol Glucosides.*

By Frederick Belding Power and Arthur Henry Salway.

The compound designated as ipuranol was first isolated in these laboratories in 1908 from the stems of *Ipomoea purpurea*, Roth (Nat. Ord. *Convolvulaceae*), received from South Africa (*Amer. J. Pharm.*, 1908, **80**, 264). The amount of substance obtained from this source was so small as to permit only of determining its empirical composition, melting point (285—290°), and the melting point of its acetyl derivative (160°). At about the same time, however, an evidently identical substance was isolated from olive bark (T., 1908, **93**, 907), and as in this instance both the substance (m. p. 285—290°) and its well-crystallised acetyl derivative (m. p. 160°) were analysed, it was concluded that ipuranol is a dihydric alcohol, possessing the formula $C_{23}H_{38}O_2(OH)_2$. The analytical figures obtained for both these compounds were, in fact, in excellent agreement with this assumption. In the same year a very small amount of a similar substance was obtained from nutmeg (*Amer. J. Pharm.*, 1908, **80**, 575), which, however, could only be obtained sufficiently pure for analysis in the form of its acetyl derivative (m. p. 163—164°). It was first in this connexion that the substance was observed to yield a colour reaction similar to that given by the phytosterols.

In the intervening years a considerable further number of substances have been isolated from widely different sources, which either agreed in composition with the formula assigned to ipuranol, or which differed from the latter in their characters and composition only to such an extent as to cause them to be regarded as homologous compounds. To these apparently related substances the following distinctive names and formulæ were assigned: citrullol, $C_{22}H_{36}O_2(OH)_2$, melting at 285—290° (T., 1910, **97**, 102), and trifolianol, $C_{21}H_{34}O_2(OH)_2$, melting at 295° (T., 1910, **97**, 249). These two substances, together with ipuranol, were thus considered to be members of a series of dihydric alcohols, represented by the general formula $C_nH_{2n-6}O_4$.

Another group of substances had meanwhile been obtained, which were likewise regarded as dihydric alcohols, and yielded colour reactions similar to those given by the phytosterols. These compounds, however, differed appreciably from the members of the ipuranol series by their greater facility of crystallisation and a generally lower melting point, as also to some extent in their percentage composition. The first member of this class of compounds was isolated from jalap tubers (*Ipomoea purga*, Hayne), and to it the name ipurganol (m. p. 222—225°) and the formula $C_{21}H_{32}O_2(OH)_2$ were respectively assigned (*J. Amer. Chem. Soc.*, 1910, **32**, 89). Various closely related substances which, together with ipurganol, appeared to constitute a series of dihydric alcohols represented by the general formula $C_nH_{2n-8}O_4$, were given the following distinctive names and formulæ: bryonol, $C_{22}H_{34}O_2(OH)_2$, melting at 210—212° (T., 1911, **99**, 943); grindelol and anonol, $C_{23}H_{36}O_2(OH)_2$, melting at 256—257° and 294—298° respectively (*Proc. Amer. Pharm. Assoc.*, 1907, **55**, 342, and *Pharm. J.*, 1911, **87**, 744); and cucurbitol, $C_{24}H_{38}O_2(OH)_2$, melting at 260° (*J. Amer. Chem. Soc.*, 1910, **32**, 36). A substance, melting at 245°, to which the name of calabarol has been given (T., 1911, **99**, 2155), was considered to possess the formula $C_{23}H_{34}O_2(OH)_2$, and therefore to be the first representative of dihydric alcohols having the general formula $C_nH_{2n-10}O_4$.

Quite recently Tutin and Clewer (T., 1912, **101**, 2230) isolated from *Cluytia similis*, Muell. Arg. (Nat. Ord. *Euphorbiaceae*), a substance which was observed to resemble ipuranol and other related compounds in its general characters, and was designated cluytianol. It was subsequently obtained in larger amount from taraxacum root (T., 1912, **101**, 2426). This substance (m. p. 300—305°) was first regarded as a trihydric alcohol, $C_{23}H_{37}O(OH)_3$ (P., 1912, **28**, 265), but a comparison of the figures obtained by its analysis and the analysis of its acetyl and benzoyl derivatives, together with a molecular-weight determination of the acetyl derivative, subsequently led the above-mentioned authors to regard cluytianol as a tetrahydric alcohol, having the formula $C_{29}H_{46}O(OH)_4$ (P., 1912,

28, 317). At the same time, and with similar considerations, ipuranol was thought to be more correctly represented as a tribydric alcohol, having the formula $C_{29}H_{47}O_2(OH)_3$, instead of $C_{23}H_{38}O_2(OH)_2$, as originally adopted.

The amounts of the various above-mentioned compounds which have hitherto been isolated were in most cases comparatively small, and, with a few exceptions, did not permit of the analysis of their derivatives. The characters of the substances nevertheless rendered it evident that they represented a new class of compounds, and as they gave well-crystallised acetyl and benzoyl derivatives, having definite melting points, it was apparent that they were of alcoholic nature. It may also be noted that, although ipuranol was first obtained from an ethyl acetate extract of the resinous constituents of the respective plant, it has subsequently, as well as the related compounds, been isolated either from the petroleum or the ether extract of the resin. In one instance (citrullol, *loc. cit.*), the substance was separated from an aqueous liquid, in which it appeared to be contained in a colloidal state. Furthermore, in a considerable number of cases the various compounds were isolated from a petroleum extract of the resinous constituents after the extract had been heated for some time with an alcoholic solution of potassium hydroxide, and this fact seemed especially to confirm the view respecting the alcoholic nature of the compounds.

It has naturally been deemed desirable to obtain, if possible, some further information regarding the character of the numerous compounds here considered, but the small amounts of material hitherto available had precluded any extended experiments in this direction. Some recent observations, however, have served to reveal the nature of the compounds in question, inasmuch as it has been found that several of them, when heated in amyl-alcoholic solution with aqueous hydrogen chloride, become hydrolysed with the formation of a phytosterol and dextrose. It is thus evident that the substances are phytosterol glucosides, and their percentage composition is also in harmony with this view.

In order that the results obtained by the analysis of these various compounds and some of their derivatives, as previously recorded, may be compared with the calculated percentage figures for phytosterol glucosides and the respective acetyl and benzoyl derivatives of the latter, they have been arranged in a tabular form, and at the same time the source of the compounds has been indicated, together with the names and formulæ hitherto assigned to them.

In addition to the list on p. 402, substances resembling ipuranol have also been obtained from the fruit of *Ecballium Elaterium*

(T., 1909, **95**, 1988) and from the bark of *Erythrophloeum Guineense* (*Amer. J. Pharm.*, 1912, **84**, 348), but not in amounts sufficient for analysis.

Name and formula.		Source.	M.p. and analysis.		M. p. and analysis of acetyl derivative.	
				C. H.		C. H.
Ipuranol,	$C_{23}H_{40}O_4$	*Ipomoea purpurea*	285—290°.	72·6; 10·6	160°.	—
,,	,,	Olive bark..................	285—290°.	72·6; 10·6	160°.	69·8; 9·4
,,	,,	Nutmeg......................	—	—	163—164°.	69·5; 9·4
,,	,,	*Prunus serotina*, bark	285—290°.	72·3; 10·7	160°.	—
,,	,,	,, ,, leaves	295°.	—	163°.	—
,,	,,	*Apocynum androsaemifolium*	285—290°.	72·4; 10·5	162°.	—
,,	,,	*Rumex Ecklonianus*	285—290°.	—	162°.	—
,,	,,	*Ornithogalum thyrsoides*	285—29 °.	72·3; 10·5	162°.	—
,,	,,	*Gelsemium sempervirens*	290°.	72·3; 10·5	162°.	—
,,	,,	*Iris versicolor*	285—290°.	72·3; 10·3	162—163°.	—
,,	,,	*Withania somnifera*	285—290°.	72·4; 10·3	164—165°.	—
,,	,,	*Buphane disticha*	286°.	—	—	—
,,	,,	*Oenanthe crocata*	285—290°.	—	161°.	—
,,	,,	*Casimiroa edulis*	280—285°.	72·6; 10·4	166°.	69·7; 9·5
,,	,,	*Ipomoea orizabensis*	285—290°.	72·4; 10·6	162°.	—
,,	,,	Scammony root..............	285—290°.	72·2; 10·6	162°.	—
Citrullol,	$C_{22}H_{38}O_4$	Colocynth	285—290°.	72·0; 10·6	167°.	69·3; 9·2
,,	,,	*Euonymus atropurpureus*....	285—290°.	71·9; 10·7	164—165°.	69·0; 9·3
,,	,,	*Caulophyllum thalictroides*..	275—280°.	71·9; 10·4	169—170°.	—
Trifolianol,	$C_{21}H_{36}O_4$	*Trifolium pratense*..........	295°.	71·4; 10·4	165—166°.	68·8; 9·2
,,	,,	,, *incarnatum*	295—300°.	71·6; 10·4	165—166°.	68·6; 9·2
Calabarol,	$C_{23}H_{36}O_4$	Calabar bean	245°.	73·1; 10·1	—	—
Ipurganol,	$C_{21}H_{34}O_4$	*Ipomoea purga*..............	222—225°.	71·7; 10·0	166—167°.	68·9; 8·8
Bryonol,	$C_{22}H_{36}O_4$	Bryony root	210—212°.	72·3; 10·1	152°.	69·1; 9·0
Grindelol,	$C_{23}H_{38}O_4$	*Grindelia camporum*	257°.	72·7; 10·2	161°.	—
Anonol,	$C_{23}H_{38}O_4$	*Anona muricata*	294—298°.	72·9; 10·3	166°.	—
Cucurbitol,	$C_{24}H_{40}O_4$	Watermelon seed	260°.	73·2; 10·4	150°.	—
Cluytianol,	$C_{29}H_{50}O_5$	*Cluytia similis*	300—305°.	72·8; 10·5	160°.	68·7; 9·1
,,	,,	Taraxacum root	297°.	72·5; 10·6	161°.	68·6; 9·1

In a few instances benzoyl derivatives of the above-mentioned compounds were prepared, which had the following characters: Benzoylipuranol, from *Oenanthe crocata* and *Casimiroa edulis*, melting at 196° and 197° respectively; benzoylanonol, m. p. 197—198°. C=75·9; H=7·8; benzoylcalabarol, m. p. 195—196°. C=76·1; H=7·8; benzoylcluytianol (from *Cluytia similis*), m. p. 192°. C=76·3; H=7·7; (from taraxacum root), m. p. 196°. C=76·2; H=7·9 per cent.

It is well known that a considerable number of isomeric phytosterols occur in nature which possess the formula $C_{27}H_{46}O$, whilst other compounds of this type are represented by the formula $C_{30}H_{50}O$ (or $C_{30}H_{48}O$) and $C_{20}H_{34}O$. As examples of these different classes there may be noted: sitosterol, $C_{27}H_{46}O$ (*Monatsh.*, 1897, **18**, 551), stigmasterol, $C_{30}H_{50}O$ or $C_{30}H_{48}O$ (*Ber.*, 1906, **39**, 4378), and rhamnol, $C_{20}H_{34}O$ (*Proc. Amer. Pharm. Assoc.*, 1904, **52**, 299). The glucosides and their acetyl and benzoyl derivatives of the first two types of phytosterols mentioned would have the following composition respectively:

Sitosterol Glucoside and Derivatives.

$C_{33}H_{56}O_6$ requires C=72·3; H=10·2 per cent.
$C_{33}H_{52}O_6(CO\cdot CH_3)_4$ requires C=68·7; H=8·9 per cent.
$C_{33}H_{52}O_6(CO\cdot C_6H_5)_4$,, C=75·9; H=7·5 ,, ,,

Stigmasterol Glucoside and Derivatives.

$C_{36}H_{60}O_6$ requires C = 73·5; H = 10·2 per cent.
$C_{36}H_{56}O_6(CO{\cdot}CH_3)_4$ requires C = 69·8; H = 9·0 per cent.
$C_{36}H_{56}O_6(CO{\cdot}C_6H_5)_4$ „ C = 76·5; H = 7·6 „ „

A comparison of these figures with those afforded by the analysis of the above-mentioned, naturally occurring compounds renders it probable that a considerable proportion of the latter are glucosides of sitosterol or one of its isomerides, whilst in some cases their composition would appear to approximate more closely to that of a stigmasterol glucoside. Inasmuch as different phytosterols are frequently found in the same plant, it is to be expected that their respective glucosides may also occur in the form of mixtures, and these would be exceedingly difficult to separate. The possibility of different sugars being united with the phytosterol complex is also not excluded.

In this connexion it may incidentally be noted that H. and A. Euler (*Ber.*, 1907, **40**, 4762) obtained from the varnish-like coating of the leaves of the common alder (*Alnus glutinosa*, Gaertn.) a substance which they designated glutinolic acid, and which was stated to give an intense cholesterol (phytosterol) reaction. To this substance, which was obtained only in an amorphous state, they assigned the formula $(C_{28}H_{48}O_5)_x$, which requires C = 72·4; H = 10·3 per cent. These figures are in such close agreement with those required for a sitosterol glucoside that, together with the colour reaction noted, it appears probable that the so-called "glutinolic acid" consisted essentially of a compound of the above-described type. The method by which the substance was obtained, together with its low melting point, likewise suggests that the observed acidic properties may have been due to its contamination with a small amount of a fatty acid.

EXPERIMENTAL.

It had previously been observed that some of the compounds under consideration were not altered by heating with aqueous hydrogen chloride in the presence of a little alcohol, but this result was evidently due to their sparing solubility, and did not preclude the possibility of their being glucosidic. As the compounds, however, were soluble in warm amyl alcohol, it was found that their hydrolysis could be effected by the use of this solvent. Such of them as were at present available in sufficient amounts have therefore now been examined in the direction indicated, and with the following results:

I. *Ipuranol* (m. p. 290—295°). A typical specimen of this substance had the following composition:

0·1147 gave 0·3039 CO_2 and 0·1084 H_2O. $C=72·3$; $H=10·5$.

The formula originally assigned to ipuranol, namely, $C_{23}H_{40}O_4$, requires $C=72·6$; $H=10·5$ per cent.

Sitosterol glucoside, $C_{33}H_{56}O_6$, requires $C=72·3$; $H=10·2$ per cent.

Half a gram of the substance was dissolved in 30 c.c. of hot amyl alcohol, and 10 c.c. of an aqueous 15 per cent. solution of hydrogen chloride added, together with sufficient ethyl alcohol to form a homogeneous liquid. After heating for two hours in a reflux apparatus, steam was passed through the mixture to remove the amyl alcohol, and the contents of the flask then filtered. A solid substance was thus collected, which, when crystallised from a mixture of alcohol and ethyl acetate, separated in glistening leaflets melting at 136°. This substance gave the phytosterol colour reaction, and it evidently belonged to that class of compounds:

0·1041 gave 0·3204 CO_2 and 0·1127 H_2O. $C=83·9$; $H=12·0$.
$C_{27}H_{46}O$ requires $C=83·9$; $H=11·9$ per cent.

The aqueous acid liquid, from which the phytosterol had been separated by filtration, was exactly neutralised with sodium carbonate, evaporated to dryness, the residue digested with absolute alcohol, and the mixture filtered. On evaporating the alcoholic filtrate a small amount of a syrupy residue was obtained, which reduced Fehling's solution, and yielded an osazone melting and decomposing at 212°. It was thus evident that the sugar consisted of dextrose.

The original compound had thus become resolved by hydrolysis into a phytosterol and dextrose, according to the equation:

$$C_{33}H_{56}O_6 + H_2O = C_{27}H_{46}O + C_6H_{12}O_6.$$

II. *Ipuranol* (m. p. 280—285°). This specimen of the substance was obtained from a different source from the preceding one, and it gave on analysis the following result:

0·0973 gave 0·2573 CO_2 and 0·0918 H_2O. $C=72·1$; $H=10·5$.
$C_{33}H_{56}O_6$ requires $C=72·3$; $H=10·2$ per cent.

Half a gram of this substance was hydrolysed in the manner above described, when it yielded a phytosterol, which separated in glistening leaflets, melting at 136°. This was analysed, and its specific rotatory power determined:

0·0911 gave 0·2800 CO_2 and 0·1002 H_2O. $C=83·8$; $H=12·2$.
$C_{27}H_{46}O$ requires $C=83·9$; $H=11·9$ per cent.

0·2649 of anhydrous substance, made up to 20 c.c. with chloroform, gave α_D $-0°56'$ in a 2-dcm. tube, whence $[\alpha]_D$ $-35·2°$.

The aqueous acid liquid obtained by the hydrolysis was found to contain a sugar, from which *d*-phenylglucosazone (m. p. 212°) was prepared.

III. *Citrullol* (m. p. 285—290°). This represented the compound which had previously been isolated from colocynth (T., 1910, **97**, 102). A small amount (0·15 gram) of its acetyl derivative was hydrolysed in the above-described manner, when it yielded a phytosterol which separated from a mixture of alcohol and ethyl acetate in small needles, melting at 145—149°. The sugar produced by the hydrolysis gave an osazone which, in the crude state, melted at 190—192°, but the amount was too small to permit of its purification.

IV. *Bryonol* (m. p. 210—212°). The compound to which this name had been assigned was obtained from bryony root (T., 1911, **99**, 943). A small amount (0·15 gram) of the substance, when hydrolysed in the manner above described, yielded a phytosterol which separated from a mixture of alcohol and ethyl acetate in leaflets, melting at about 90—100°. When recrystallised from alcohol it was obtained in small tufts of needles, which sintered at 90° and melted completely at 105°. The same melting point was observed after it had been kept in a vacuum desiccator for forty-eight hours. The phytosterol dissolved completely in concentrated sulphuric acid with a yellow colour and green fluorescence, and evidently differed from sitosterol. A small amount of sugar was likewise produced by the hydrolysis, and this was found to reduce Fehling's solution, but it was not sufficient for the preparation of an osazone.

V. *Cluytianol* (m. p. 297°). The specimen of this compound which served for its present examination was obtained from taraxacum root (T., 1912, **101**, 2426). A small amount (0·2 gram) of the substance was hydrolysed as above described, when it yielded a phytosterol which crystallised in glistening leaflets, melting at 136°. The sugar produced by the hydrolysis was found to reduce Fehling's solution, but was not sufficient in amount for the preparation of an osazone.

Although it has not been possible to subject all the compounds enumerated in the preceding list to the above-described treatment, the results which are now recorded are deemed quite sufficient to lead to the conclusion that they are all phytosterol glucosides. Such compounds had not previously been known to occur in nature, and it may be regarded as of some significance that in practically all instances where their isolation had been effected phytosterols of varying character had also been found. With consideration of the facts which have thus been elicited respecting the nature of the compounds in question, it is not deemed desirable that distinctive names should be perpetuated for them, but that they should receive some collective designation, and it is therefore proposed that they be termed *phytosterolins*. The future examination of compounds of this type, when available in sufficient amounts, would appear to be most suitably directed to the characterisation of the phytosterols which they yield on hydrolysis, and also, when possible, to that of the sugar produced.

It may finally be noted that some recent experiments, conducted by one of us, have led to the synthesis of sitosterol-*d*-glucoside, which has been found to possess properties agreeing with those of the substance designated as ipuranol. The details of these experiments will form the subject of a further communication.

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