696. *Vitexin. Part I.*

By W. H. Evans, A. McGookin, L. Jurd, Alexander Robertson, and W. R. N. Williamson.

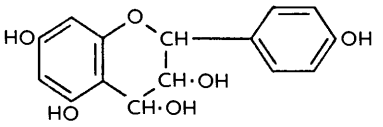
Vitexin, isolated from puriri wood, is shown to be a lævorotatory compound, $C_{21}H_{20}O_{10}$. Since its trimethyl ether on oxidation gave 8-formyltri-*O*-methylapigenin, it consists of an apigenin nucleus with a $\cdot C_6H_7O(OH)_4$ residue attached at the 8-position. Parallel with this the hydrolytic fission of tri-*O*-methylvitexin gave di-*O*-methyl*apo*vitexin which is shown to be 2-hydroxy-4 : 6-dimethoxyacetophenone with a $\cdot C_6H_7O(OH)_4$ residue in the 3-position. Oxidation of vitexin with periodic acid or sodium metaperiodate gives dehydro*seco*vitexin from which warm methanolic sulphuric acid removes D-glyceraldehyde as the dimethyl acetal, leaving a mixture of two isomeric di-*O*-methyl derivatives. Tentative structures for vitexin and the above degradation products are advanced.

The yellow pigment, vitexin, along with homovitexin, from New Zealand puriri wood (*Vitex littoralis*), was first examined by Perkin [1] who proposed the empirical formula $C_{15}H_{14}O_7$ or $C_{17}H_{16}O_8$, prepared a polyacetate, and showed that on hydrolytic decomposition with alkali the compound gave phloroglucinol, *p*-hydroxybenzoic acid, and *p*-hydroxyacetophenone. Further, Perkin noted that vitexin gave the same degradation products as apigenin and formed a tetranitro-derivative which he subsequently showed to be tetranitroapigenin [2] in agreement with his earlier suggestion that vitexin might consist of an apigenin nucleus with a side-chain attached. To accommodate his results Perkin [2] revised the empirical formula for vitexin to $C_{21}H_{20}O_{10}$ and suggested that the compound was a very stable glucoside of apigenin with an abnormal mode of attachment of the sugar residue. Subsequently, Barger [3] examined Perkin's vitexin which he claimed was a product of the acid hydrolysis of saponarin, being formed along with saponaretin considered to be impure homovitexin. On the basis of molecular-weight determinations and acetyl values Barger concluded that vitexin had the empirical formula $C_{15}H_{14}O_7$ and was represented by structure (I) or (II); in a personal communication to Barger [3] Perkin suggested a third possibility (III), a conclusion supported later by Péteri.[4] On the other hand Nakaoki [5] claimed that the glycoside saponarin gives Barger's saponaretin on hydrolysis and this is then transformed by longer treatment with hot dilute acids into vitexin which is an optical isomeride of saponaretin. Further, Nakaoki concluded that vitexin had formula $C_{21}H_{22}O_{10}$ and contained alcoholic and phenolic hydroxyl groups, forming an octa-acetyl derivative. On fusion with alkali the hydriodic

[1] Perkin, *J.*, 1898, **73**, 1019.
[2] Perkin, *J.*, 1900, **77**, 422.
[3] Barger, *J.*, 1906, **89**, 121.
[4] Péteri, *J.*, 1939, 1635.
[5] Nakaoki, *J. Pharm. Soc. Japan*, 1944, **64**, No. 11*A*, 51; *Chem. Abs.*, 1952, **46**, 108.

acid reduction product of vitexin gave *n*-hexanoic acid whilst on oxidation with permanganate vitexin furnished a substance which appeared to be 2 : 4 : 6-trihydroxyphenylacetic acid. Consequently Nakoaki concluded that vitexin was an apigenin derivative having a 2 : 3 : 4 : 5 : 6-pentahydroxy-*n*-hexyl residue attached at the 6- or 8-position, *e.g.*, formula (V; R = H, R′ = $C_6H_{13}O_5$). In connexion with the problem of the vitexin precursor it is of interest that Geissman and Kranen-Fielder [6] isolated glycosides of vitexin which do not give saponaretin as an intermediate product of hydrolysis.

On isolation from puriri wood by means of acetone without the intervention of mineral acids vitexin, m. p. 264—265°, has been found to be lævorotatory and is not appreciably racemised by prolonged boiling with 5% hydrochloric acid. From the analytical results obtained for the compound and for the numerous derivatives and degradation products now described it is clear that vitexin has the empirical formula $C_{21}H_{20}H_{10}$, as originally proposed by Perkin.[2] The compound does not contain a *C*-methyl group and on acetylation with boiling acetic anhydride and sodium acetate forms a hepta-acetate having a negative ferric reaction, whilst by the pyridine method a penta-acetate is formed, exhibiting a positive ferric reaction. Treatment of the penta-acetate with an excess of diazomethane furnished tetra-*O*-acetyldi-*O*-methylvitexin with a positive ferric reaction and giving di-*O*-methylvitexin on deacetylation. With methyl iodide and potassium carbonate in boiling acetone the penta-acetate gave rise to tetra-*O*-acetyltri-*O*-methylvitexin which on deacetylation yielded tri-*O*-methylvitexin with a negative ferric reaction; with ethyl iodide the corresponding tetra-*O*-acetyltri-*O*-ethylvitexin was obtained. By the Purdie method the trimethyl ether gave rise to a mixture of penta- and hexa-*O*-methylvitexin. The infrared absorption spectra of hepta-*O*-acetyl- and tetra-*O*-acetyltri-*O*-methyl-vitexin indicate the absence of a free hydroxyl group whereas the spectrum of the hexamethyl ether shows hydroxyl absorption.



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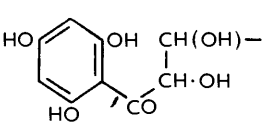

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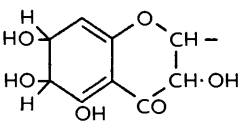

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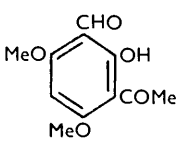

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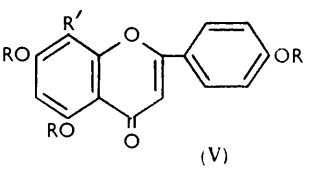

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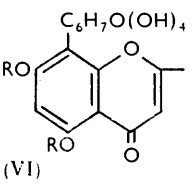


(VI)

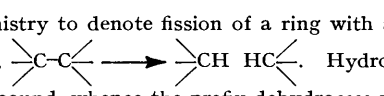
Hydrolytic decomposition of tri-*O*-methylvitexin with hot aqueous sodium hydroxide gave *p*-methoxyacetophenone and *p*-anisic acid but with boiling aqueous barium hydroxide an additional product, di-*O*-methyl*apo*vitexin $C_{14}H_{16}O_7(OMe)_2$, was obtained; the analogous di-*O*-ethyl*apo*vitexin has also been prepared. Both compounds gave ferric reactions and formed acyl derivatives. With an excess of aqueous periodic acid di-*O*-methyl*apo*vitexin gave rise to 3-formyl-4 : 6-di-*O*-methylphloracetophenone (IV) which was identified by reduction to 3-methyl-4 : 6-di-*O*-methylphloracetophenone and by comparison with a synthetic specimen. In this and similar periodate oxidations of vitexin and its derivatives the production of formaldehyde and a volatile acid fraction was observed but the amounts formed appeared to be too small to be significant. The formation of the 3-formylphloracetophenone residue clearly arises by the removal of a hydroxylated C_5-residue. Similarly, the oxidation of tri-*O*-methylvitexin with lead tetra-acetate

[6] Geissman and Kranen-Fielder, *Naturwiss.*, 1956, **43**, 226.

or by warm dilute nitric acid removed the same residue, giving 8-formyltri-*O*-methylapigenin (V; R = Me, R′ = CHO) which formed an oxime and a 2 : 4-dinitrophenylhydrazone and on reduction with a Raney nickel catalyst furnished 5 : 7 : 4′-trimethoxy-8-methylflavone (V; R = R′ = Me), identical with a synthetic specimen. From these degradations of di-*O*-methyl*apo*- and tri-*O*-methyl-vitexin, it is clear that tri-*O*-methylvitexin and consequently vitexin contain a C_6-system at the 8-position of the apigenin residue and may be represented by (V; R = Me) and (V; R = H) respectively. Further, of the ten oxygen atoms five are in the apigenin residue and are not concerned in the attachment of the C_6-system, because, from its conversion into 8-formyltri-*O*-methylapigenin, tri-*O*-methylvitexin contains a tri-*O*-methylapigenin residue. The remaining five oxygen atoms are contained in the C_6-moiety and, since hepta-*O*-acetyl- and tetra-*O*-acetyltri-*O*-methyl-vitexin do not contain a free hydroxyl group, this unit contains four active hydroxyl groups and an inert oxygen atom and consequently may be written $\cdot C_6H_7O(OH)_4$. This indifferent oxygen atom is not in a carbonyl group since vitexin does not show carbonyl activity and the infrared absorption spectra of tri- and hexa-*O*-methylvitexin indicated that the only carbonyl group present was conjugated in the nucleus, *i.e.*, the carbonyl of the apigenin residue. Therefore in the absence of a lactone or ester group it seemed likely that the fifth oxygen of the C_6-residue is in all probability present in an ether system and hence tri-*O*-methylvitexin and vitexin may be represented by the partial structure (VI; R = Me) and (VI; R = H) respectively.

To define the orientation of the hydroxyl system present in the C_6-residue a study of the oxidation of vitexin with varying quantities of periodic acid and sodium metaperiodate was undertaken. With aqueous periodic acid the oxidation of vitexin and its derivatives appeared to be abnormal; an excess of the reagent produced a comparatively rapid, initial reaction until about 1·5—2 mol. of periodic acid were used, followed by a gradually slowing oxidation but the results were somewhat variable. In all cases the initial stages of the oxidation were consistent with 1 : 2-glycol fission but ultimately traces of formaldehyde and small amounts of volatile acid were formed with further oxidation, *e.g.*, formation of 8-formylapigenin. The reaction did not, however, provide evidence for the presence of a glycerol system in the vitexin side-chain. With sodium metaperiodate in place of periodic acid the results were almost equally variable and again indicated that the reaction is not confined to a smooth fission of a 1 : 2-glycol system. The small amounts of formaldehyde were not regarded as significant and hence the C_6-residue did not appear to contain the system $\cdot CH(OH)\cdot CH_2\cdot OH$. It may well be that in addition to the production of 8-formylapigenin side reactions occur involving the phenolic apigenin residue (cf. the behaviour of phenols with periodic acid [7]).

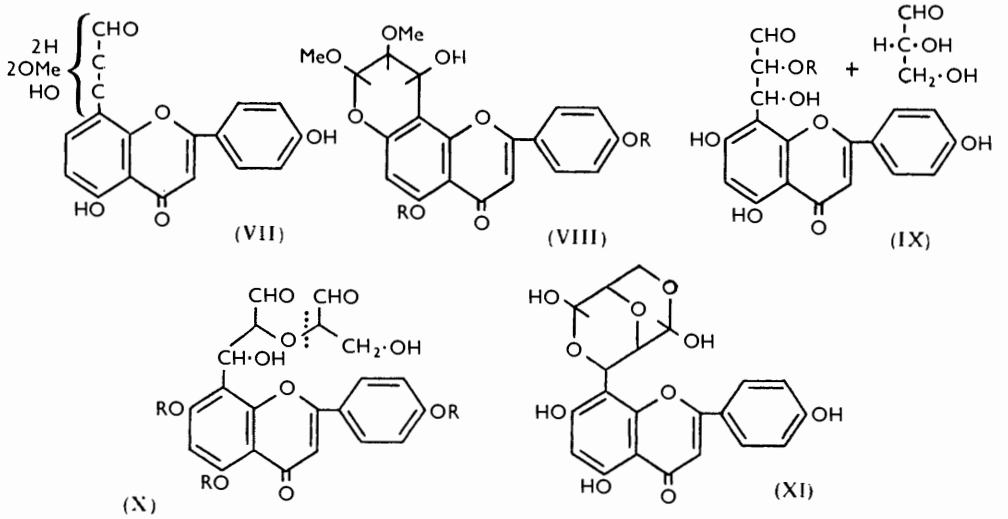
From the oxidation of methanolic vitexin with periodic acid or, more successfully, with sodium metaperiodate there readily separated a sparingly soluble, lævorotatory, yellow solid accompanied by small amounts of 8-formylapigenin. The composition of this product $C_{21}H_{18}O_{10}$ accords with oxidative fission of a cyclic 1 : 2-glycol system and accordingly it has been named dehydro*seco*vitexin.* Under similar conditions the oxidation of tri-*O*-methyl- and di-*O*-methyl*apo*-vitexin was somewhat erratic and the glycol scission products, dehydrotri-*O*-methyl*seco*- and dehydrodi-*O*-methyl*secoapo*-vitexin, which were somewhat difficult to purify, were accompanied by 8-formyltri-*O*-methylapigenin and 3-formyl-2-hydroxy-4 : 6-dimethoxyacetophenone respectively. The more satisfactory yields of dehydro*seco*vitexin may be due to the deposition of this product from the reaction mixture whereas the products from vitexin trimethyl ether and

* The prefix *seco* was introduced in steroid chemistry to denote fission of a ring with addition of a hydrogen atom at each carbon atom affected, *viz.*,  . Hydrogen is not added in the conversion of vitexin into the new compound, whence the prefix dehydro*seco* seems more appropriate.

[7] Pennington, *J. Amer. Chem. Soc.*, 1947, **69**, 187.

apo-vitexin dimethyl ether remain dissolved and are isolated with solvents; somewhat improved yields were obtained when one molecular proportion of oxidising agent was employed. Although dehydro*seco*vitexin reduced Fehling's solution and ammoniacal silver nitrate, it failed to form carbonyl derivatives and gave an infrared spectrum devoid of absorption in the aldehyde region, a result not entirely unexpected in the case of hydroxylated scission products from 1 : 2-cyclic glycols (cf. oxidation of 2 : 5-anhydro-D-mannitol [8]). Dehydro*seco*vitexin forms a penta-acetate with a negative ferric reaction and a penta-*p*-nitrobenzoate whilst dehydrotri-*O*-methyl*seco*vitexin gives a di-*p*-nitrobenzoate. Unlike vitexin or its ethers, dehydro*seco*-, dehydrotri-*O*-methyl*seco*-, and dehydrodi-*O*-methyl*secoapo*-vitexin are sensitive to acids. With a warm solution of 2 : 4-dinitrophenylhydrazine in dilute sulphuric acid these compounds gave a precipitate of the bis-2 : 4-dinitrophenylhydrazone of pyruvaldehyde which was also formed, more slowly, from penta-*O*-acetyldehydro*seco*vitexin under the same conditions. Distillation of these degradation compounds with dilute acids gave a distillate containing pyruvaldehyde but in alkaline media volatile carbonyl compounds were not formed. Further, with hot methanolic sulphuric acid dehydro*seco*vitexin gave in addition to pyruvaldehyde a mixture of two optically active crystalline products $C_{18}H_{12}O_6(OMe)_2$, A and B, which are readily separated by fractional crystallisation.

With regard to the source of the pyruvaldehyde derived from dehydro*seco*vitexin, it became clear, since vitexin is devoid of a *C*-methyl group, that this product was an artefact, the most probable source of which appeared to be the action of the mineral acid on glyceraldehyde or dihydroxyacetone, *i.e.*, $HO{\cdot}CH_2{\cdot}CH(OH){\cdot}CHO$ or $HO{\cdot}CH_2{\cdot}CO{\cdot}CH_2{\cdot}OH \longrightarrow CH_3{\cdot}CO{\cdot}CHO + H_2O$. From a mixture obtained by heating dehydro*seco*vitexin with methanolic sulphuric acid a small amount of a neutral colourless oil was isolated which on treatment with warm acidic 2 : 4-dinitrophenylhydrazine yielded the bis-2 : 4-dinitrophenylhydrazone of pyruvaldehyde. With *p*-nitrobenzoyl chloride and pyridine this oil gave a di-*p*-nitrobenzoate found to be identical with the di-*p*-nitrobenzoate of D-glyceraldehyde dimethyl acetal; the corresponding diethyl acetal derivative of the



precursor was also prepared. Accordingly, therefore, it is clear that the scission of *seco*-vitexin with acids gives, in addition to the compounds A and B, D-glyceraldehyde, the formyl group of which is clearly produced by the scission of a 1 : 2-glycol system of vitexin with sodium metaperiodate or periodic acid.

With methyl iodide–potassium carbonate the compounds A and B form dimethyl

[8] Bera, Foster, and Stacey, *J.*, 1956, 4531.

ethers, $C_{18}H_{10}O_4(OMe)_4$, insoluble in aqueous sodium hydroxide and having negative ferric reactions. The infrared absorption spectra of each ether exhibits a hydroxyl band. In agreement with these compounds A and B form tri-*p*-nitrobenzoates, and the dimethyl ether of compound A or B gives a mono-*p*-nitrobenzote, thus confirming the presence of three hydroxyl groups in these degradation products.

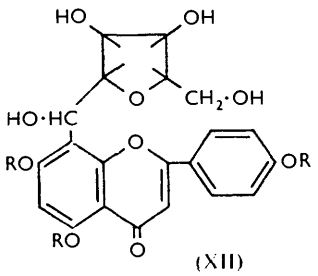
From the composition of compounds A and B, each of which contains two methoxyl groups, and from the ultraviolet absorption spectra of their dimethyl ethers it seemed reasonably certain that compounds A and B contained an apigenin residue retaining the three-carbon residue left after the removal of the glyceraldehyde moiety from dehydro-*seco*vitexin. Further, in vew of the formation of dehydro*seco*vitexin by periodic acid oxidation, this residual C_3 unit attached to the apigenin residue at the 8-position would be expected to contain an aldehyde or potential aldehyde group and, *inter alia*, could be written as a second glyceraldehyde residue as in formula (VII). From the properties of compounds A and B, including ferric reaction, solubilities in alkali, and methylation by a procedure which effects the alkylation of phenolic hydroxyl groups only (cf. methylation of phloridzin [9]), it seems clear that the hydroxyl groups at the 5- and the 4′-positions of the compounds are free and that the C_3 residue attached at the 8-position contains two methoxyl groups acquired during the treatment of dehydro*seco*vitexin with methanolic sulphuric acid; experiments have established that vitexin and 7-hydroxyflavone are not methylated by hot methanolic sulphuric acid. Further, the 7-hydroxyl group of the apigenin unit must be concerned in the attachment of this C_3 residue otherwise it would undergo methylation by potassium carbonate–methyl iodide simultaneously with the hydroxyl groups at the 5- and the 4′-position. Moreover, since the dimethyl ethers of compounds A and B contain a free alcoholic (and not phenolic) hydroxyl group and form *p*-nitrobenzoates the C_3 residue contains an alcoholic hydroxyl group. Thus compounds A and B are formed from dehydro*seco*vitexin by the loss of a glyceraldehyde residue with the simultaneous cyclisation and methylation of the residual hydroxylated side chain (containing a second glyceraldehyde residue). Of the possible expressions derived from (VII) the hemiacetal structure (VIII; R = H) appears best to accommodate the properties of compounds A and B, with (VIII; R = Me) representing their respective dimethyl ethers. This permits of the existence of two optical isomerides, which could be separated by fractional crystallisation and would arise by the cyclisation of an optically active glyceraldehyde residue (IX; R = H) corresponding to formation of the methyl α- and β-glycosides. In agreement with the structures proposed, compounds A and B reduce Fehling's solution after treatment with hot mineral acids.

The immediate precursor of compounds A and B may be considered to be (IX; R = Me) and consequently dehydro*seco*vitexin may be derived by the union of (IX; R = H) with a glyceraldehyde residue. From this a number of formulæ are possible for dehydro-*seco*vitexin, of which we regard (X; R = H) as the most acceptable. This structure accommodates the hydroxyl groups, the indifferent oxygen atom of vitexin retained in dehydro*seco*vitexin, and the aldehyde or potential aldehyde groups arising by glycol fission. Although the behaviour of vitexin and certain derivatives towards periodic acid and metaperiodate are somewhat abnormal there is no evidence of a glycerol scission with quantitative elimination of formic acid, or of the scission of the system ·CH(OH)·CH_2·OH with the elimination of more than traces of formaldehyde. That dehydro*seco*vitexin does not show carbonyl reactivity may well be due to its existence as a hemiacetal system of which several forms are possible, *e.g.*, (XI) (cf. sugar derivatives [8]). Further, in the scission of the oxide system in dehydro*seco*vitexin with methanolic sulphuric acid leading to the formation of compounds A and B the reaction would take place as indicated by the broken line in (X) with methylation of the precursor of compound A, to give (IX; R = Me) which would then cyclise and furnish the " glycoside " (VIII; R = H). Clearly this degradation of dehydro*seco*vitexin is facilitated by the activating carbonyl groups; the

[9] Müller and Robertson, *J.*, 1933, 1170.

ready methanolysis invoked is comparable in some respects with the *O*-alkylation of α-hydroxy-ketones.[10] Consequently, vitexin and its trimethyl ether may be represented by formula (XII; R = H) and (XII; R = Me) respectively. This structure accounts for the optical activity, and preparation of phenolic trialkyl ethers and a hepta-acetate.

Whilst at present we regard the expression (XII; R = H) for vitexin as the best summation of our experimental results a number of alternative structures have been considered in detail, of which only (XIII) with dehydro*seco*vitexin as (XIV) need be mentioned now. Although formula (XIII) is in keeping with (*a*) the slight reduction of Fehling's solution by vitexin, (*b*) Nakaoki's claim to have isolated 2 : 4 : 6-trihydroxy-phenylacetic acid, and (*c*) to some extent the behaviour of vitexin and tri-*O*-methylvitexin towards an excess of periodic acid and sodium metaperiodate, it appears to be excluded for the following reasons. Vitexin does not show carbonyl activity or form an osazone expected from (XIII) and is not methylated with methanolic sulphuric acid (cf. formation of fructosides); it only slowly reduces hot Fehling's solution and this is not unexpected in view of the behaviour of piscidic acid.[11] The residue (XV) left after the removal of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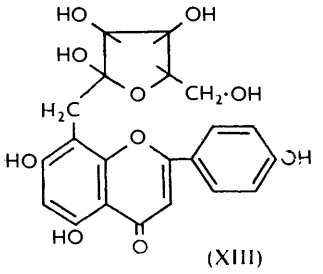
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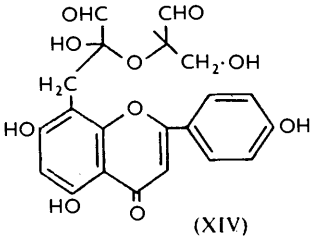
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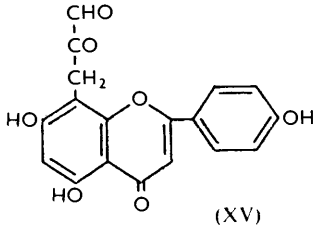
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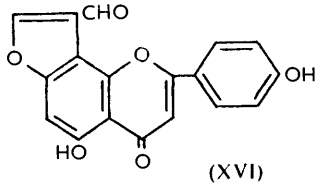
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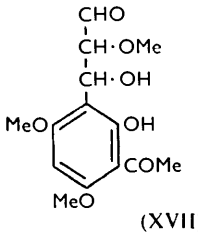
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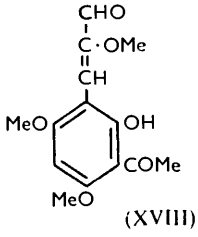
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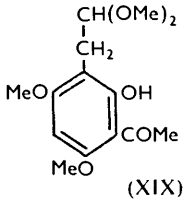
(XIX)

glyceraldehyde from a dehydro*seco*vitexin having formula (XIV) would be optically inactive and with hot methanolic sulphuric acid would be expected to cyclise to an inactive benzofuran derivative (XVI).

The behaviour of di-*O*-methyl*apo*vitexin with periodic acid or sodium metaperiodate follows that of vitexin, resulting in the formation of dehydrodi-*O*-methyl*secoapo*vitexin; the rate of oxidation varies somewhat and the initial reaction was followed by a slower

[10] Bergmann and Gierth, *Annalen*, 1926, **448**, 48.
[11] Bridge, Coleman, and Robertson, *J.*, 1948, 257.

reaction with the absorption of varying amounts of reagent. With hot methanolic sulphuric acid this compound gives rise to pyruvaldehyde, probably by way of D-glyceraldehyde, and an optically inactive compound $C_{10}H_8O_2(OMe)_4$ which gives a ferric reaction and, from its composition and infrared absorption spectrum, appears to be the dimethyl acetal (XIX) of 3-acetyl-2-hydroxy-4 : 6-dimethoxyphenylacetaldehyde. If this view proves correct it seems likely that, after the removal of the D-glyceraldehyde moiety from dehydrodi-*O*-methyl*secoapo*vitexin, the residual system (XVII) undergoes rearrangement in the acid media with an intermediate (XVIII) from which extrusion of the formyl group occurs leaving a residue. This in acidic media re-arranges to give a phenylacetaldehyde (XIX) (cf. Zincke [12]). The ferric reaction given by this compound precludes a dicyclic system involving the hydroxy-group in the *ortho*-position to the acetyl residue of dehydrodi-*O*-methyl*secoapo*vitexin; the hydrogen bonding occurring in (XVII) would tend to inhibit the cyclisation to a substance corresponding to compound A.

Experimental

Vitexin.—This compound was isolated by Perkin's method,[1] and several modifications of it, but the following procedure gave superior yields and was adopted in large-scale work; the various methods gave identical products.

Finely milled wood of *Vitex littoralis* (20 kg.) was extracted in a continuous extractor with boiling acetone for 20 hr. and the concentrated extract (15 l.) decanted from a tar (A) (220 g.). On being concentrated to *ca.* 5 l. and kept at 0° for 24 hr. the extract deposited a crystalline solid (B) (52 g.) which was washed with hot acetone. The filtrate and washings from this were evaporated and on being kept a solution of the residual gum in boiling methanol (3 l.) deposited a greenish-yellow solid (C) (45 g.) which was collected and washed with warm acetone. The tar (A) was boiled with two portions of alcohol (each, 600 ml.), and the residual greenish-yellow solid (D) (25 g.) collected. The methanolic and alcoholic filtrates from (D) and (C), respectively, were combined, concentrated to 1·5 l., diluted with hot water (15 l.) and hydrochloric acid (1·5 l.), and boiled for 1 hr. After being decanted from a black tar which had separated and was discarded, the clear, orange solution was boiled for 5 hr. On cooling, this slowly deposited crude vitexin (E) (125 g.) in 24 hr.; a further quantity (F) (63·5 g.) separated in about 3 weeks.

The solids (B) (52 g.), (C) (45 g.), and (D) (25 g.) were combined, washed several times with boiling alcohol and then acetone, and recrystallised from 80% aqueous dioxan, giving vitexin in glistening yellow plates (83 g.), m. p. 264—265°, $[\alpha]_D^{20}$ −14·50° (*c* 2·789 in pyridine), $\lambda_{max.}$ 270 and 332 mμ (log ε 4·225 and 4·240), $\lambda_{min.}$ 248 and 282 mμ (log ε 3·949 and 4·011), which had not been in contact with mineral acid and gave a red ferric reaction in alcohol (Found: C, 58·4, 58·4, 58·6; H, 4·6, 4·8, 4·7. Calc. for $C_{21}H_{20}O_{10}$: C, 58·3; H, 4·7. Calc. for $C_{15}H_{14}O_7$: C, 58·8; H, 4·6. Calc. for $C_{21}H_{22}O_{10}$: C, 58·1; H, 5·1%).

Washed and recrystallised in the same manner, the combined solids (E) (125 g.) and (F) (63·5 g.) gave vitexin (132 g.), m. p. 264—265°, $[\alpha]_D^{20}$ −14·35° (*c* 2·414 in pyridine), identical with the foregoing specimen. Prolonged heating of either sample in 4 or 5% hydrochloric acid did not affect the specific rotation. The total yield of vitexin was 215 g. or 1·075% of wood.

Microcrystalline or pulverised vitexin is soluble in methanol, alcohol, acetone, or ethyl acetate and was readily soluble in pyridine and in hot dioxan, glycerol, phenol, or *cyclo*hexanone; larger crystals of the compound dissolve remarkably slowly.

Acetylation of vitexin (0·2 g.) with acetic anhydride (2 ml.) and sodium acetate (0·5 g.) at 100° for 1·5 hr. or with pyridine (5 ml.) and acetyl chloride (2 g.) on the steam-bath for 3 hr. gave the hepta-acetate which separated from acetone–methanol in colourless prisms or from acetic acid–alcohol in rhombs, m. p. 257—258°, $[\alpha]_D^{20}$ −73·2° (*c* 2·074 in acetone), with a negative ferric reaction in alcohol [Found: C, 57·9; H, 4·9; OAc, 41·3, 42·7. Calc. for $C_{21}H_{13}O_3(OAc)_7$: C, 57·9; H, 4·7; OAc, 41·5%].

A solution of vitexin (10 g.) in pyridine (100 ml.) and acetic anhydride (100 ml.) was heated on the steam-bath for 2 hr. and then slowly diluted with hot 2N-aqueous acetic acid (*ca.* 750 ml.) until crystals began to separate. On cooling, the mixture deposited the *penta-acetate* which crystallised from aqueous pyridine or methanol in faintly yellow needles; this derivative gave a deep red ferric reaction and melted at 146—147°, resolidified, and then melted again at 247°

[12] Zincke, *Annalen*, 1882, **216**, 298.

(decomp.), $[\alpha]_D^{20}$ −4·43° (*c* 2·559 in acetone) [Found, in a specimen dried in a vacuum at 100° (mean of 5 analyses): C, 58·0; H, 4·8; Ac, 33·5. $C_{21}H_{15}O_5(OAc)_5$ requires C, 57·9; H, 4·7; Ac, 33·5%].

Tetra-O-acetyldi-O-methylvitexin.—On the addition of an excess of ethereal diazomethane to a solution of vitexin penta-acetate, m. p. 247° (1 g.), in methanol (5 ml.) a vigorous reaction ensued, the solution became red, and a yellow compound separated. The resulting *tetra-O-acetyldi-O-methylvitexin* separated from aqueous methanol and then absolute methanol in yellow needles, m. p. 205—206°, $[\alpha]_D^{20}$ −13·52° (*c* 1·08 in acetone), which gave a red ferric reaction [Found: C, 59·3; H, 5·2; OMe, 9·5. $C_{29}H_{26}O_{12}(OMe)_2$ requires C, 59·2; H, 5·1; OMe, 9·9%].

Ammonia (5 ml.; *d* 0·88) was added to this acetate (1 g.) in methanol (60 ml.) and 18 hr. later the solution was concentrated and gave *di-O-methylvitexin*, forming yellow needles from methanol with a deep red ferric reaction; on being heated it melted at 182°, solidified, and then melted at 264° [Found, in a specimen dried in a high vacuum: C, 59·8; H, 5·5; OMe, 12·5. $C_{21}H_{18}O_8(OMe)_2$ requires C, 60·0; H, 5·3; OMe, 13·5%]. With acetic anhydride–pyridine on the steam-bath for 2 hr. this compound, which is soluble in cold 2N-aqueous sodium hydroxide, regenerates the tetra-acetate, m. p. and mixed m. p. 205°.

Tetra-O-acetyltri-O-methylvitexin.—Methylation of the penta-acetate (4·15 g.), m. p. 247°, with potassium carbonate (40 g.) and methyl iodide (40 ml.) in boiling acetone (100 ml.) for 8 hr. gave a gum. A solution of this in warm pyridine was treated with hot water until solid began to separate and, on cooling, the mixture deposited a product which gave a faint red ferric reaction. Repetition of the methylation process with this material gave *tetra-O-acetyltri-O-methylvitexin*, forming from methanol colourless prisms (12·1 g.), m. p. 202°, $[\alpha]_D^{20}$ −9·82° (*c* 2·139 in acetone), which had a negative ferric reaction and on being heated melted at 202°, solidified, and then melted at 212°; a stable form, m. p. 212°, was occasionally obtained [Found: C, 59·5; H, 5·3; OMe, 13·9; OAc, 25·8. $C_{21}H_{13}O_3(OMe)_3(OAc)_4$ requires C, 59·8; H, 5·3; OMe, 14·5; OAc, 26·8%].

In boiling acetone (50 ml.) the interaction between vitexin penta-acetate (3 g.) and an excess of methyl iodide and potassium carbonate for 4 hr. gave a gum which on crystallisation from aqueous pyridine gave tetra-*O*-acetyldi-*O*-methylvitexin (1·3 g.), m. p. 198—199°, which, twice recrystallised from methanol, formed yellow needles, m. p. and mixed m. p. 204—205°, $[\alpha]_D^{20}$ −13·3° (*c* 1·626 in acetone), having a red ferric reaction. Addition of water to the aqueous-pyridine liquor precipitated a little tetra-*O*-acetyltri-*O*-methylvitexin which separated from aqueous pyridine and then methanol in prisms, m. p. and mixed m. p. 200—201°.

Deacetylation of tetra-*O*-acetyltri-*O*-methylvitexin (12·5 g.) in hot methanol (500 ml.) with ammonia (40 ml.; *d* 0·88) gave tri-*O*-methylvitexin as a *dihydrate* which was isolated from the concentrated reaction mixture, washed with acetone, and crystallised from methanol, forming colourless needles (8·5 g.), m. p. 288°, with a negative ferric reaction, insoluble in cold aqueous sodium hydroxide [Found: C, 56·4; H, 6·0; OMe, 17·8; OAc, negative. $C_{21}H_{17}O_7(OMe)_3,2H_2O$ requires C, 56·5; H, 5·9; OMe, 18·2%]. Acetylation of this ether by acetic anhydride–pyridine regenerated tetra-*O*-acetyltri-*O*-methylvitexin, m. p. and mixed m. p. 202° and 212°.

A mixture of tri-*O*-methylvitexin (0·1 g.), *p*-nitrobenzoyl chloride (0·3 g.), and pyridine (0·4 ml.) was heated on the steam-bath for ½ hr. and poured into dilute hydrochloric acid. The solid was triturated with aqueous sodium hydrogen carbonate, washed, and crystallised from methanol and then acetone–methanol, giving the *tetra*-p-*nitrobenzoate* of tri-*O*-methylvitexin in colourless feathery needles, m. p. 176°, with a negative ferric reaction [Found: N, 5·2, 5·3; OMe, 9·1. $C_{49}H_{29}O_{19}N_4(OMe)_3$ requires N, 5·4; OMe, 8·9%].

Tri-O-ethylvitexin.—Vitexin acetate (6·5 g.), m. p. 247°, was heated with ethyl iodide (20 ml.) and potassium carbonate (10 g.) in boiling acetone (150 ml.) for 16 hr. and the gummy product isolated and crystallised from aqueous pyridine and then methanol. This solid was again heated with ethyl iodide (10 ml.) and potassium carbonate (5 g.) in acetone (50 ml.) for 5 hr. Crystallised from aqueous pyridine and then methanol, the resulting *tetra-O-acetyltri-O-ethylvitexin* formed colourless prisms (3·9 g.), m. p. 236°, with a negative ferric reaction (Found: C, 61·4; H, 5·4. $C_{35}H_{40}O_{14}$ requires C, 61·4; H, 5·9%). Deacetylation of this compound (3·5 g.) with ammonia (15 ml.; *d* 0·88) in methanol (100 ml.) at room temperature for 24 hr. gave *tri-O-ethylvitexin* which separated from methanol in colourless needles (2·6 g.), m. p. 270°, having a negative ferric reaction (Found, in a specimen dried in a high vacuum: C, 63·0; H, 6·0. $C_{27}H_{32}O_{10}$ requires C, 62·8; H, 6·2%).

In one experiment the tetra-*O*-acetyltri-*O*-ethylvitexin was accompanied by a small amount

of a compound which appeared to be a *tetra*-O-*acetyldi*-O-*ethylvitexin* which separated first on the gradual addition of water to a solution of the reaction product in hot pyridine. Recrystallised several times from aqueous pyridine and then methanol, the diethyl derivative formed clusters of yellow needles, m. p. 187·5°, with an intense red-brown ferric reaction (Found: C, 59·8; H, 5·6. $C_{33}H_{36}O_{14}$ requires C, 60·3; H, 5·5%).

Penta- and Hexa-O-*methylvitexin*.—A mixture of tri-*O*-methylvitexin (11 g.), silver oxide (25 g.), methyl iodide (40 g.), and acetone (500 ml.) was heated under reflux for 50 hr. The gum left on evaporation of the filtered mixture was heated in boiling methyl iodide (40 ml.), containing silver oxide (15 g.), with the addition of more iodide (40 ml.), for 24 hr. On isolation, the gummy product was dissolved in boiling benzene (100 ml.), and the hot solution slowly treated with light petroleum (b. p. 40—60°) until it became faintly opalescent. After having been kept at room temperature for 2 hr., the liquor was decanted from a little oil which had separated, heated to the b. p., and treated with more light petroleum (40 ml.). A little more oil then separated and this process was repeated, a crystalline solid ultimately separating. This was removed and the mother-liquor poured into an excess of boiling light petroleum (b. p. 40—60°), giving when cold more crystalline solid. The combined solid fractions were washed with benzene, leaving crude *hexa*-O-*methylvitexin* (1·6 g.), m. p. 190—200°, which on purification from benzene–light petroleum (b. p. 60—80°) and then benzene, formed glistening colourless needles, m. p. 205°, $[\alpha]_D^{20}$ +13·45° (*c* 1·784 in MeOH), with a negative ferric reaction [Found: C, 62·7; H, 6·6; OMe, 36·8. $C_{21}H_{14}O_4(OMe)_6$ requires C, 62·8; H, 6·2; OMe, 36·0%].

The benzene washings of the crude hexamethyl ether were added to the combined oils which had been precipitated first with light petroleum and on being kept the solution deposited more hexamethyl ether which was isolated. Concentration of the benzene liquor then gave *penta*-O-*methylvitexin* (1 g.), m. p. 220°, which was boiled with benzene to remove traces of the hexamethyl ether, and then crystallised from from ethyl acetate–acetone, forming colourless glistening prisms, m. p. 234°, with a negative ferric reaction [Found: C, 62·2; H, 6·2; OMe, 32·0. $C_{21}H_{15}O_5(OMe)_5$ requires C, 62·1; H, 6·0; OMe, 30·9%].

By fractionation of the residues from benzene and light petroleum more crude penta- (0·6 g.), m. p. 220°, and hexa-methyl ether (1·7 g.), m. p. 190°, were obtained.

A mixture of penta-*O*-methylvitexin (50 mg.), *p*-nitrobenzoyl chloride (0·15 g.), and pyridine (1 ml.) was heated on the steam-bath for 30 min., cooled, and poured into dilute hydrochloric acid. After having been triturated with aqueous sodium hydrogen carbonate and washed with water, the solid was crystallised from acetone–methanol, giving the p-***nitrobenzoate*** of penta-*O*-methylvitexin in colourless glistening prisms, m. p. 277° [Found: C, 60·1; H, 5·3; N, 2·2, 2·3; OMe, 26·2. $C_{28}H_{18}O_8N(OMe)_5$ requires C, 60·8; H, 5·1; N, 2·2; OMe, 25·3%].

Hydrolytic Fission of Tri-O-*methylvitexin*.—(*a*) A mixture of tri-*O*-methylvitexin (2 g.) and 8% aqueous sodium hydroxide (100 ml.) in nitrogen was boiled until a homogeneous solution was formed. A current of steam was then led into the boiling solution, and the distillate (150 ml.) collected. A small portion of the distillate was treated with aqueous 2 : 4-dinitrophenylhydrazine sulphate, giving a copious precipitate of *p*-methoxyacetophenone 2 : 4-dinitrophenylhydrazone which, on purification by chromatography from benzene on aluminium oxide followed by crystallisation from ethylene glycol or ethyl acetate, had m. p. and mixed m. p. 256° (0·38 g.) (Found: N, 17·0. Calc. for $C_{15}H_{14}O_5N_4$: N, 17·0%). From the remainder of the distillate *p*-methoxyacetophenone was isolated with ether and converted into the semicarbazone (0·46 g.), m. p. and mixed m. p. 198° (Found: C, 57·8; H, 6·3; N, 20·0. Calc. for $C_{10}H_{13}O_2N_3$: C, 58·0; H, 6·3; N, 20·3%).

The brown solid obtained by acidification of the residual alkaline liquor was crystallised from methanol, giving *p*-anisic acid (0·23 g.), m. p. and mixed m. p. 182° (Found: C, 63·3; H, 5·4. Calc. for $C_8H_8O_3$: C, 63·2; H, 5·3%), which formed the amide, m. p. and mixed m. p. 164° (Found: N, 9·4. Calc. for $C_8H_9O_2N$: N, 9·3%).

(*b*) A rapid stream of nitrogen was passed through boiling saturated aqueous barium hydroxide (200 ml.), containing tri-*O*-methylvitexin (2 g.), for 2 hr. and the effluent gas led into aqueous 2 : 4-dinitrophenylhydrazine, giving a precipitate of *p*-methoxyacetophenone 2 : 4-dinitrophenylhydrazone (0·08 g.), m. p. 256°. The hot homogeneous alkaline liquor was acidified with dilute sulphuric acid, cooled, and filtered and the solid extracted with boiling methanol, giving *p*-anisic acid (0·55 g.), m. p. 182°. The filtrate was treated with a little barium carbonate, filtered, and evaporated in a vacuum at 40°, leaving a pale yellow product which on crystallisation from dioxan gave *di*-O-*methyl*apo*vitexin* in rosettes of colourless needles (0·95 g.), m. p.

ca. 137°. After repeated purification from alcohol–light petroleum (b. p. 60—80°) or from acetone, this compound melted at 126—130°, solidified, and then melted at 222° (decomp.), having $[\alpha]_D^{20}$ +4·59° (*c* 1·417 in MeOH) [Found: C, 53·2; H, 6·3; OMe, 16·8. $C_{14}H_{16}O_7(OMe)_2$ requires C, 53·6; H, 6·2; OMe, 17·3%]. This neutral product was soluble in chloroform, acetic acid, pyridine, or warm alcohol and gave a deep red ferric reaction in alcohol and an emerald-green colour with Gibbs's reagent.

A mixture of di-*O*-methyl*apo*vitexin (50 mg.), *p*-nitrobenzoyl chloride (0·2 g.), and pyridine (1 ml.) was heated on the steam-bath for ½ hr. On isolation the *penta*-p-*nitrobenzoate* formed almost colourless needles, m. p. 192°, from acetone–methanol, with a negative ferric reaction [Found: C, 54·9; H, 3·7; N, 6·2; OMe, 5·6. $C_{49}H_{31}O_{22}N_5(OMe)_2$ requires C, 55·5; H, 3·4; N, 6·4; OMe, 5·6%]. Acetylated with acetic anhydride and sodium acetate, di-*O*-methyl*apo*vitexin gave an acetate which separated from light petroleum in colourless needles, m. p. 151—152°, with a negative ferric reaction.

3-*Formyl*-2-*hydroxy*-4 : 6-*dimethoxyacetophenone*.—A solution of 6% aqueous para-periodic acid (10 ml.) was added to di-*O*-methyl*apo*vitexin (0·2 g.) in acetic acid (10 ml.), and the mixture agitated for 5 hr., diluted with water (100 ml.), neutralised with a slight excess of sodium hydrogen carbonate, and then kept for 16 hr. On isolation the yellow deposit was digested with hot methanol (10 ml.) and, on cooling, the extract deposited 3-formyl-2-hydroxy-4 : 6-dimethoxyacetophenone in pale yellow-green tablets (0·13 g.), m. p. and mixed m. p. 170° (Found: C, 58·6; H, 5·3. Calc. for $C_{11}H_{12}O_5$: C, 58·9; H, 5·4%). The 2 : 4-dinitrophenylhydrazone separated from acetic acid in orange-red needles, m. p. 259°, identical with a synthetical specimen.

The aqueous liquors left after the separation of the crude 3-formyl-2-hydroxy-4 : 6-dimethoxyacetophenone contained formaldehyde, isolated as the 2 : 4-dinitrophenylhydrazone (85 mg.), m. p. and mixed m. p. 158°.

Synthetic 3-formyl-2-hydroxy-4 : 6-di-*O*-methoxyacetophenone was prepared by the following modification of Gruber and Traub's method.[13] The solid formed by the interaction of 2-hydroxy-4 : 6-dimethoxyacetophenone (5 g.), hydrogen cyanide (10 ml.), and aluminium chloride (6 g.) in ether (150 ml.), saturated at 0° with hydrogen chloride, during 24 hr., was dissolved in water (250 ml.), and the solution almost neutralised with 2N-aqueous sodium hydroxide. On being kept at 80° for 45 min. this gave the formyl-ketone which separated from alcohol in greenish-yellow needles or from ethyl acetate in tablets (5·4 g.), m. p. 170°. The 2 : 4-*dinitrophenylhydrazone* separated from acetic acid in red needles, m. p. 259° (Found: N, 14·2. $C_{17}H_{16}O_8N_4$ requires N, 14·0%).

Reduction of 3-formyl-2-hydroxy-4 : 6-dimethoxyacetophenone (4 g.) with zinc amalgam (10 g.) in acetic acid (30 ml.) and concentrated hydrochloric acid (8 ml.) for 2—3 min. and dilution of the filtered mixture with water gave 2-hydroxy-4 : 6-dimethoxy-3-methylacetophenone [14] (2·5 g.), m. p. and mixed m. p. 144°, after purification from alcohol.

Hydrolytic Fission of Tri-O-*ethylvitexin*.—Tri-*O*-ethylvitexin (2 g.) was heated in boiling saturated aqueous barium hydroxide (100 ml.) for 3 hr. and the cooled mixture treated with carbon dioxide, filtered, and evaporated in a vacuum. From the residue boiling methanol extracted a gum which on crystallisation from dioxan, containing a little ether, gave *di*-O-*ethyl*apo*vitexin* which on recrystallisation from acetone formed pale yellow needles, m. p. 203·5—204°, with a red ferric reaction in alcohol [Found: C, 56·1; H, 6·9; OEt, 22·9. $C_{14}H_{16}O_7(OEt)_2$ requires C, 55·9; H, 6·8; OEt, 23·3%]. The *penta*-p-*nitrobenzoate* separated from acetone–methanol in almost colourless needles, m. p. 175°, with a negative ferric reaction [Found: C, 55·7; H, 3·8; N, 6·2; OEt, 8·3. $C_{49}H_{31}O_{22}N_5(OEt)_2$ requires C, 56·2; H, 3·7; N, 6·2; OEt, 8·0%].

Oxidation of Tri-O-*methylvitexin with Lead Tetra-acetate*.—(*a*) A mixture of the ether (1·5 g.), lead tetra-acetate (4·5 g.), and acetic acid (25 ml.) was kept at 25° for 5 days, poured into water (100 ml.), and extracted with chloroform. The combined chloroform extracts from 5 experiments were washed with 2N-aqueous sodium hydrogen carbonate, dilute aqueous sodium hydroxide, and then water, dried, and evaporated, leaving a yellow product (2·5 g.) which was triturated thrice with acetone (3 ml.). Crystallised from alcohol, the residue gave 8-*formyl*-5 : 7 : 4′-*trimethoxyflavone* in pale yellow plates (0·45 g.), m. p. 237° (decomp.), with a negative ferric reaction [Found: C, 66·6; H, 5·1; OMe, 25·4. $C_{16}H_7O_3(OMe)_3$ requires C, 67·0; H, 4·7;

[13] Gruber and Traub, *Monatsh.*, 1947, **77**, 414.
[14] Curd and Robertson, *J.*, 1933, 437.

OMe, 27·4%]. The 2 : 4-dinitrophenylhydrazone was an unstable red solid, m. p. 250—254° (decomp.). By the hydroxylamine–pyridine method the formylflavone gave a derivative which had the composition of a *dioxime*, forming almost colourless needles, m. p. 232° (decomp.), from methanol (Found: C, 61·8; H, 5·6; N, 7·2. $C_{19}H_{18}O_6N_2$ requires C, 61·6; H, 4·9; N, 7·6%). Treatment of the aqueous liquors left after precipitation of the 8-formyl-5 : 7 : 4′-trimethoxyflavone with 2 : 4-dinitrophenylhydrazine and with dimedone gave the 2 : 4-dinitrophenylhydrazone, m. p. and mixed m. p. 159°, and the dimedone derivative, m. p. and mixed m. p. 188°, of formaldehyde.

Reduction of 8-formyl-5 : 7 : 4′-trimethoxyflavone (0·4 g.) with hydrogen at 60 lb./sq. in. and Raney nickel W.5 catalyst [15] in dioxan (50 ml.) gave a product, which on repeated crystallisation from benzene–light petroleum (b. p. 60—80°) formed rosettes of colourless needles (0·15 g.), m. p. 228°, undepressed on admixture with a specimen of synthetic 5 : 7 : 4′-trimethoxy-8-methylflavone, m. p. 230°, prepared by the following method.

A mixture of 2-hydroxy-4 : 6-dimethoxy-3-methylacetophenone (13 g.), *p*-anisoyl chloride (12 g.), and pyridine (75 ml.) was heated on the steam-bath for 3 hr., cooled, and poured into water (500 ml.). Crystallised from 95% alcohol and washed, the resulting solid gave 2-p-*anisoyloxy*-4 : 6-*dimethoxy*-3-*methylacetophenone* in needles (16 g.), m. p. 169° (Found: C, 65·7; H, 5·9. $C_{19}H_{20}O_6$ requires C, 66·3; H, 5·9%. Heated with pulverised sodamide (35 g.) in boiling benzene (75 ml.) for 4 hr. this keto-ester (7 g.) gave 2-*hydroxy*-4 : 6 : 4′-*trimethoxy*-3-*methyldibenzoylmethane* which was separated from the crude reaction product with aqueous sodium hydroxide and recovered by acidification of the extracts with acetic acid and isolation with chloroform. Crystallised from alcohol and then acetone–light petroleum (b. p. 60—80°), the diketone formed yellow-green needles (4 g.), m. p. 184° (decomp.), with an emerald-green ferric reaction (Found: C, 66·6; H, 5·6. $C_{19}H_{20}O_6$ requires C, 66·3; H, 5·9%). The *monoxime* separated from alcohol in colourless needles, m. p. 188° (Found: N, 4·0. $C_{19}H_{21}O_6N$ requires N, 3·9%). Cyclised with 75% sulphuric acid (30 ml.) at room temperature in 10 min., this diketone (2·5 g.) gave 5 : 7 : 4′-*trimethoxy*-8-*methylflavone* which separated from benzene in needles (2·3 g.), m. p. 230° (decomp.) (Found: C, 70·4; H, 5·3. $C_{19}H_{18}O_5$ requires C, 69·9; H, 5·5%). The natural and the synthetic specimen had identical ultraviolet absorption spectra.

(*b*) Oxidation of tri-*O*-methylvitexin (1·5 g.) was effected with lead tetra-acetate (4·5 g.) in acetic acid (20 ml.) at 18° for 4 days and the mixture poured into water (150 ml.). The resinous, brownish-yellow precipitate from three experiments was washed with water and dissolved in methanol (20 ml.). On being kept for a week this solution deposited the trimethyl ether of dehydro*seco*vitexin (0·7 g.), m. p. 194° (decomp.), which on recrystallisation from methanol–pyridine formed tiny pale yellow needles, m. p. 201—202° (decomp.), identical with the product obtained by periodic acid oxidation.

Oxidation of Tri-O-methylvitexin with Nitric Acid.—A mixture of tri-*O*-methylvitexin (0·3 g.), concentrated nitric acid (5 ml.), and water (25 ml.) was heated under reflux for 1·5 hr., cooled, and filtered to remove a little *p*-anisic acid, m. p. and mixed m. p. 180°, which had separated. The filtrate was basified with a slight excess of 2N-aqueous sodium hydroxide and extracted with chloroform (15 ml. × 4). Evaporation of the dried extracts left 8-formyl-5 : 7 : 4′-trimethoxyflavone which separated from alcohol in needles, m. p. and mixed m. p. 236°.

Oxidation of Tri-O-methylvitexin with Periodic Acid.—(*a*) 0·5N-Periodic acid (35 ml.) was added to a solution of the ether (1 g.) in methanol (150 ml.), and the mixture kept in the dark for 10 hr., filtered, and neutralised (phenolphthalein) with aqueous barium hydroxide. On being evaporated to 50 ml. the filtered solution deposited an intractable resin which was removed, and the liquor was extracted with chloroform (50 ml. × 3). Evaporation of the combined dried extracts left a yellow solid (0·1 g.) which was triturated with acetone (5 ml.) and crystallised from alcohol, giving 8-formyl-5 : 7 : 4′-trimethoxyflavone in pale yellow needles (50 mg.), m. p. and mixed m. p. 235°; in another experiment under similar conditions 7·5 g. of trimethyl ether gave 0·45 g. of the formylflavone.

(*b*) 0·5N-Aqueous periodic acid (70 ml.) was added in portions of 10 ml. at intervals of 1 hr. to a stirred solution of tri-*O*-methylvitexin (2 g.) in acetic acid (40 ml.). The mixture was diluted with excess of saturated aqueous sodium hydrogen carbonate at 35° and kept at room temperature for 4 days. The resulting precipitate was washed with water, dried, and extracted with boiling methanol (15 ml.). On being kept the extract slowly deposited *dehydrotri*-O-*methyl*seco*vitexin* (0·9 g.), m. p. 197—198°, which on recrystallisation from pyridine–methanol or much

[15] Mozingo, *Org. Synth.*, 1941, **21**, 15.

methanol had m. p. 201—202° [Found: C, 61·4, 61·0, 60·8; H, 5·4, 5·7, 5·7; OMe, 20·8. $C_{21}H_{15}O_7(OMe)_3$ requires C, 61·0; H, 5·1; OMe, 19·7%].

The use of sodium metaperiodate in place of periodic acid gave the same product, m. p. and mixed m. p. 200° (1·3 g. from 6 g. of tri-*O*-methylvitexin). With either reagent the yield was variable.

Oxidation of Tri-O-methylvitexin with Sodium Metaperiodate in Aqueous Methanol at Room Temperature.—

Experiment 1.

Time	25 min.	45 min.	113 min.	16 hr.
$NaIO_4$, mols.	1·77	2·64	3·97	5·38

Experiment 2.

Time	20 min.	32 min.	47 min.	61 min.	115 min.
$NaIO_4$, mols.	1·13	1·63	1·73	1·92	2·14

On being distilled, a solution of the dehydrotri-*O*-methyl*seco*vitexin (0·2 g.) in a mixture of methanol (10 ml.), water (10 ml.), and concentrated sulphuric acid (2 ml.) gave a distillate containing pyruvaldehyde which was isolated as the bis-2 : 4-dinitrophenylhydrazone, m. p. and mixed m. p. 299—300° (Found: C, 41·7; H, 3·1; N, 25·2. Calc. for $C_{15}H_{12}O_8N_8$: C, 41·7; H, 2·8; N, 25·9%), and as the bisphenylhydrazone which separated from dilute alcohol in pale yellow needles, m. p. 145° (Found: C, 71·3; H, 6·5; N, 22·0. Calc. for $C_{15}H_{16}N_4$: C, 71·4; H, 6·4; N, 22·2%).

The *di*-p-*nitrobenzoate* of the dehydrotri-*O*-methyl*seco*vitexin separated from acetone–methanol in almost colourless needles, m. p. 159—160° (Found: N, 3·7. $C_{38}H_{30}O_{16}N_2$ requires N, 3·6%). Acetylation of the trimethyl ether (0·2 g.) with pyridine (5 ml.) and acetic anhydride (1·5 ml.) gave a *product* which separated from benzene–light petroleum (b. p. 60—80°) in colourless needles, m. p. 94° [Found: C, 60·6; H, 4·4. $C_{28}H_{28}O_{12}$ (diacetate) requires C, 60·4; H, 5·1. $C_{28}H_{26}O_{11}$ (anhydro-diacetate) requires C, 62·4; H, 4·8%].

*Dehydroseco*vitexin.—A solution of vitexin (8·3 g.) was prepared by heating an excess of vitexin in boiling methanol (1 l.) containing water (300 ml.) for 2 hr. The filtered solution was quickly cooled and treated with sodium metaperiodate (15 g.) in water (1 l.), and the mixture diluted with water to 4·5 l. The solid which had separated from this during 20 hr. was isolated, washed with water and then acetone, and recrystallised from aqueous pyridine and then from much methanol–acetone, giving *dehydroseco*vitexin in pale yellow prisms (4·2 g.), m. p. >360°, $[\alpha]_D^{20}$ −147° (*c* 1·0 in pyridine) (Found, in a specimen dried at 105°: C, 58·5; H, 4·5. $C_{21}H_{18}O_{10}$ requires C, 58·6; H, 4·2%). This compound had a deep red ferric reaction in alcohol, rapidly reduced Fehling's solution and gave a silver mirror with ammoniacal silver nitrate at 50—60° in *ca.* 30 min. With acetic anhydride–pyridine it gave a *penta-acetate* which separated from 50% acetic acid and then acetone–methanol in colourless needles, m. p. 242°, $[\alpha]_D^{20}$ −68·25° (*c* 0·7325 in acetic acid), with a negative ferric reaction (Found: C, 58·0, 58·2; H, 4·4, 4·7. $C_{31}H_{28}O_{15}$ requires C, 58·1; H, 4·4%). The *penta*-p-*nitrobenzoate* crystallised from acetone–methanol in pale brownish needles, m. p. 225° (Found: N, 6·0. $C_{56}H_{33}O_{25}N_5$ requires N, 6·0%).

Dehydro*seco*vitexin was also obtained by adding a solution of sodium metaperiodate (10 g.) in water (300 ml.) to a slurry of vitexin (10 g.) in alcohol (300 ml.) and stirring the mixture for 24 hr.; the purified compound (6·35 g.) had $[\alpha]_D^{20}$ −146·5° (*c* 0·7165 in pyridine). In a series of experiments in daylight at room temperature it was found that after 17 hr., various amounts of sodium periodate were consumed, *e.g.*, 1·50, 1·69, 1·85, 2·07, 2·60, 3·11, 3·25 mols. In the absence of light at 0° the corresponding amounts varied from 1·08 to 1·19 mols.

The aqueous filtrate and washings from crude dehydro*seco*vitexin were concentrated in a vacuum and repeatedly extracted with ether. Evaporation of the combined washed and dried extracts gave 8-*formylapigenin* which separated from methanol in slender yellow needles (0·628 g.), m. p. 301°, and was optically inactive (Found, in material dried in a high vacuum: C, 64·0; H, 3·5. $C_{16}H_{10}O_6$ requires C, 64·4; H, 3·4%). This compound, which had a deep red ferric reaction, formed an acetate, m. p. 176°, and on methylation by methyl iodide–potassium carbonate in boiling acetone gave a small yield of 8-formyl-5 : 7 : 4′-trimethoxyflavone, m. p. and mixed m. p. 236°.

A solution of dehydro*seco*vitexin (0·1 g.) in methanol (10 ml.) and concentrated sulphuric

acid (10 ml.) was distilled with the addition of water (10 ml.) during the distillation, and the distillate treated with aqueous 2 : 4-dinitrophenylhydrazine sulphate. Crystallised from dioxan, the precipitate gave the bis-2 : 4-dinitrophenylhydrazone of pyruvaldehyde, m. p. and mixed m. p. 299—300° (Found: N, 25·2. Calc. for $C_{15}H_{12}O_8N_8$: N, 25·9%). With phenylhydrazine the distillate gave the bisphenylhydrazone of pyruvaldehyde, forming pale yellow needles, m. p. and mixed m. p. 145° (Found: N, 22·0. Calc. for $C_{15}H_{16}N_4$: N, 22·2%).

The same results were obtained when the compound was heated with dioxan and concentrated hydrochloric acid.

Compounds A and B.—(*a*) A mixture of dehydro*seco*vitexin (10·1 g.), absolute methanol (350 ml.), and concentrated sulphuric acid (10 ml.) was heated under reflux for 2·5 hr., the cooled, clear yellow solution was diluted with ether (1500 ml.), and the ethereal solution separated and extracted several times with aqueous sodium hydrogen carbonate. Acidification of the washings followed by treatment with aqueous 2 : 4-dinitrophenylhydrazine sulphate gave the bis-2 : 4-dinitrophenylhydrazone of pyruvaldehyde. The semisolid material left on evaporation of the dried ether solution was dissolved in much acetone and the solution concentrated until solid began to separate and cooled. On isolation the product was recrystallised several times from acetone, giving *compound A* in pale yellow prisms (1·1 g.), m. p. above 360°, $[\alpha]_D^{20}$ +90·5° (*c* 1·1 in pyridine) [Found: C, 62·4, 62·1; H, 4·9, 5·0; OMe, 15·8, 15·9. $C_{18}H_{12}O_6(OMe)_2$ requires C, 62·2; H, 4·7; OMe, 16·1%]. The *tri*-p-*nitrobenzoate* of this compound separated from acetone in almost colourless needles, m. p. 277° (Found: N, 5·1. $C_{41}H_{27}O_{17}N_3$ requires N, 5·0%). Prepared by methyl iodide–potassium carbonate, the *dimethyl ether* formed colourless needles, m. p. 247—249°, $[\alpha]_D^{20}$ +86·3° (*c* 0·0183 in MeOH), from acetone and then methanol, having a negative ferric reaction [Found: C, 64·0; H, 5·6; OMe, 29·3. $C_{18}H_{10}O_4(OMe)_4$ requires C, 63·8; H, 5·4; OMe, 29·9%]. The p-*nitrobenzoate* of this dimethyl ether crystallised from methanol in pale yellow needles, m. p. 145° (Found: N, 2·5. $C_{29}H_{25}O_{11}N$ requires N, 2·5%).

The acetone filtrate from crude compound A was mixed with ethyl acetate and the greater part of the acetone distilled. On being kept the residue deposited *compound B* (3·5 g.) which crystallised from ethyl acetate in yellow prisms, decomposing at 188—190° after sintering at 178°, $[\alpha]_D^{20}$ −118° (*c* 3·5 in pyridine), with a red ferric reaction [Found, in a specimen dried at room temperature: C, 59·9; H, 5·1; OMe, 14·4, 14·4. $C_{18}H_{12}O_6(OMe)_2,H_2O$ requires C, 59·4; H, 5·0; OMe, 15·3. Found, in a specimen dried in a high vacuum at 135—150°: C, 62·1; H, 4·8. $C_{20}H_{18}O_8$ requires C, 62·2; H, 4·7%]. Tha *tri*-p-*nitrobcnzoatc* formed pale brown needles, m. p. 254—255° (decomp.), from acetone (Found: N, 5·1; OMe, 7·4. $C_{40}H_{24}O_{16}N_3{\cdot}OMe$ requires N, 5·0; OMe, 7·5%). The *deimthyl ether* of compound B separate from acet one and then methanol in colourless needles, m. p. 251—252°, $[\alpha]_D^{20}$ −68·5° (*c* 0·206 in MeOH), with a negative ferric reaction [Found: C, 63·7, 63·4; H, 5·8, 5·7; OMe, 29·1. $C_{18}H_{10}O_4(OMe)_4$ requires C, 63·8; H, 5·4; OMe, 29·9%]. The *p*-nitrobenzoate of this dimethyl ether crystallised from methanol in almost colourless needles, m. p. 124—126° (Found: N, 2·5. $C_{29}H_{25}O_{11}N$ requires N, 2·5%).

(*b*) A mixture of dehydro*seco*vitexin (2·0 g.), methanol (80 ml.), and concentrated sulphuric acid (2·0 ml.) was heated under reflux for 2 hr. and the cooled clear yellow solution poured into water containing an excess of barium carbonate. ½ Hour later the filtered mixture was treated with more barium carbonate, filtered, and evaporated in a vacuum with the addition of several portions of benzene (50 ml.) during the evaporation. The residue was twice extracted with warm acetone and the filtered extracts were evaporated, leaving a viscous oil, a sample of which gave the bis-2 : 4-dinitrophenylhydrazone of pyruvaldehyde with the acidic reagent. After being washed with ether this product was dissolved in a little pyridine and treated with excess of *p*-nitrobenzoyl chloride on the steam-bath for 1·5 hr. On isolation in the usual manner the crude solid was triturated with aqueous sodium hydrogen carbonate, washed, dried, and extracted with hot ligroin (b. p. 100—120°). Crystallised from the same solvent and then from methanol, and finally aqueous methanol, the extract gave the *bis*-p-*nitrobenzoate* of D-glyceraldehyde dimethyl acetal in clusters of needles, m. p. 104—106°, $[\alpha]_D^{18}$ +59·6° (*c* 0·054 in $CHCl_3$) Found, in a specimen dried in a high vacuum at 80°: C, 52·5; H, 4·3; N, 6·8; OMe, 13·3. $C_{17}H_{12}O_8N_2(OMe)_2$ requires C, 52·5; H, 4·2; N, 6·5; OMe, 14·3%] (yield, 0·3 g. from 2 g. of *seco*vitexin); admixture with an authentic specimen, m. p. 107—108°, did not depress the m. p. of this bis-*p*-nitrobenzoate; the infrared absorption spectra of the compounds from the two sources were identical. In another experiment with dehydro*seco*vitexin (2 g.) the aqueous

distillate was collected and poured into acidic 2 : 4-dinitrophenylhydrazine, giving a precipitate of pyruvaldehyde bis-2 : 4-dinitrophenylhydrazone (0·5 g.), m. p. 304°, after purification from acetic acid. The residue from the distillation gave the bis-*p*-nitrobenzoate of glyceric aldehyde dimethyl acetal, m. p. 104—106°.

A specimen of the synthetic bis-*p*-nitrobenzoate of D-glyceraldehyde dimethyl acetal[16] was prepared from D-glyceraldehyde dimethyl acetal by the pyridine method. The compound separated from aqueous methanol in clusters of colourless needles, m. p. 107—108°, $[\alpha]_D^{18}$ +71·73° (*c* 0·590 in $CHCl_3$) (Found: C, 52·4; H, 4·3; N, 6·7; OMe, 14·9%).

(*c*) The D-glyceraldehyde diethyl acetal was isolated from dehydro*seco*vitexin according to method (*b*) and converted into the *di*-p-*nitrobenzoate* of D-glyceraldehyde diethyl acetal which separated from methanol in clusters of needles, m. p. 97·5—98° (Found, in a specimen dried in a vacuum at 80°; C, 54·4; H, 5·0. $C_{21}H_{22}O_{10}N_2$ requires C, 54·5; H, 4·8%).

In the course of exploratory experiments DL-glyceraldehyde dimethyl and diethyl acetate were prepared and converted into the respective di-*p*-nitrobenzoates. The *di*-p-*nitrobenzoate* of the dimethyl acetal separated from methanol or ligroin (b. p. 100—120°) in clusters of needles, m. p. 82—83° [Found: C, 52·8; H, 4·1; N, 6·9; OMe, 14·0. $C_{17}H_{12}O_8N_2(OMe)_2$ requires C, 52·5; H, 4·2; N, 6·5; OMe, 14·3%]. The *di*-p-*nitrobenzoate* of the diethyl acetal formed needles, m. p. 117°, from ligroin [Found: C, 54·5; H, 4·6; N, 6·4; OEt, 19·3. $C_{17}H_{12}O_8N_2(OEt)_2$ requires C, 54·5; H, 4·8; N, 6·1; OEt, 19·5%].

*Degradation of Di-*O*-methyl*apo*vitexin.*—This compound (0·5 g.) in water (100 ml.) was treated with a solution of sodium metaperiodate (0·32 g.) in water (50 ml.). Next day the solution, which gave a negative reaction with starch–iodide paper, was concentrated in a vacuum, treated with twice its volume of acetone, filtered, and again concentrated. The concentrate was evaporated with the addition of benzene–acetone, and a solution of the residue in benzene containing a little acetone and ethyl acetate was decanted from a little insoluble material and kept at room temperature for five days. *Dehydrodi-*O*-methyl*secoapo*vitexin* gradually separated in prisms (0·34 g.), m. p. 155—158° with sintering at *ca.* 145° and on recrystallisation from benzene–acetone–ethyl acetate–methanol (10 : 7 : 3 : 6) formed needles which on being heated softened to a translucent gum at 158—159° and then melted with darkening at 179—180°, $[\alpha]_D^{23}$ +30·7° (*c* 1·640 in MeOH) [Found: C, 53·8; H, 5·5; OMe, 18·0. $C_{14}H_{14}O_7(OMe)_2$ requires C, 53·9; H, 5·6; OMe, 17·4%]. In alcohol the compound gave a faint purple ferric reaction which was intensified on the addition of a little water.

*Oxidation of Di-*O*-methyl*apo*vitexin with Sodium Metaperiodate in Aqueous Methanol at Room Temperature.*—

Experiment 1.				
Time	21 min.	54 min.	70 min.	120 min.
$NaIO_4$, mols.	0·71	1·0	1·11	1·20
Experiment 2.				
Time	8 min.	25 min.	60 min.	180 min.
$NaIO_4$, mols.	1·16	1·46	1·58	1·71

Dehydrodi-*O*-methyl*secoapo*vitexin (0·72 g.) was heated in boiling methanol (32·3 ml.) containing concentrated sulphuric acid (0·82 ml.) for ¾ hr. and the cooled solution poured into a mixture of methanol (100 ml.), water (25 ml.), and barium carbonate (4 g.). The filtered mixture was evaporated in a vacuum, leaving a solid (0·31 g.), m. p. 95—100°, which on purification from benzene or aqueous methanol gave the *dimethyl acetal* of 3-acetyl-2-hydroxy-4 : 6-dimethoxyphenylacetaldehyde in pale yellow needles, m. p. 114—116°, having a purple ferric reaction in alcohol [Found: C, 59·2, 59·3, 59·4; H, 7·4, 7·5, 7·1; OMe, 42·1, 43·1, 42·9. $C_{10}H_8O_2(OMe)_4$ requires C, 59·1; H, 7·1; OMe, 43·6%]. This product was accompanied by pyruvaldehyde which was isolated as the bis-2 : 4-dinitrophenylhydrazone.

UNIVERSITY OF LIVERPOOL. [*Received, March 30th*, 1957.]

[16] Fischer and Baer, *J. Biol. Chem.*, 1937, **128**, 466.