485. *Constituents of* Vitex Agnus Castus *Seeds. Part I. Casticin.**

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" Vitexin," isolated by Malet [1] from *Vitex agnus castus*, has now been obtained pure and shown to differ from a flavone obtained from *Vitex lucens* and also designated vitexin. The name casticin is proposed for the compound from *Vitex agnus castus*.

Casticin has been shown to be a quercetagetin derivative, 5,3′-dihydroxy-3,6,7,4′-tetramethoxyflavone.

From *Vitex agnus castus*, long known as a medicinal plant,[2] Malet [1] in 1903 obtained two crystalline substances, vitexin and vitexinine. In our investigation of *Vitex agnus castus* seeds a light petroleum extract deposited a crystalline yellow substance, $C_{19}H_{18}O_8$. Its infrared absorption spectrum indicated the presence of hydroxyl and carbonyl groups and aromatic rings, and the ultraviolet absorption was similar to those of the flavones.[3] Moreover, our substance gave several colour reactions characteristic of flavones.[4]

Our flavone is probably identical with the vitexin obtained by Malet, the crystalline form and colour reactions being the same, though from the melting point and analyses his sample was probably impure. The name vitexin is now being used for the flavone obtained from *Vitex lucens*,[5] so we propose the name casticin for the flavone from *Vitex agnus castus* seeds.

Analytical results (C, H, O, and methoxyl) indicate that casticin is a dihydroxytetramethoxyflavone, and it yields a diacetate and a diethyl ether. Exhaustive methylation with dimethyl sulphate yielded a hexamethyl derivative, which proved to be quercetagetin hexamethyl ether. With hydriodic acid casticin afforded a hexahydroxy-derivative, whose ultraviolet absorption spectrum was essentially that reported by Briggs and Locker [6] for quercetagetin and whose hexa-acetate was identical with authentic hexa-*O*-acetylquercetagetin. Thus casticin is a tetramethyl ether of quercetagetin.

Alkaline hydrolysis of casticin and di-*O*-ethylcasticin gave isovanillic and 3-ethoxy-4-methoxybenzoic acid, respectively, so that casticin contains a free 3′-hydroxyl group. Reduction with magnesium and zinc in acid solution [4] gave a crimson colour which indicated a flavone with a 3-methoxyl group. Since the Wilson [4] test was positive, the second free hydroxyl group is probably in position 5. Nevertheless, casticin, artemetin (5-hydroxy-3,6,7,3′,4′-pentamethoxyflavone),[7] and quercetagetin, gave in Hörhammer and Müller's [8] test a yellow colour but no yellow fluorescence with zirconium oxide dichloride as would be expected from a 5-hydroxyflavone. Partial methylation with

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[1] Malet, " Étude botanique et chimique du Vitex Agnus-Castus," Thesis, Montpellier, 1903.
[2] Madaus, " Lehrbuch der biologischen Heilmittel," Leipzig, Georg Thieme Verlag, 1938, p. 441.
[3] Skarzynski, *Biochem. Z.*, 1939, **301**, 150.
[4] Geissmann, " Moderne Methoden der Pflanzenanalyse," ed. Paech and Tracey, Springer-Verlag, Berlin, 1955, Vol. III, p. 467.
[5] Briggs and Cambie, *Tetrahedron*, 1958, **3**, 269.
[6] Briggs and Locker, *J.*, 1951, 3136.
[7] Čekan and Herout, *Coll. Czech. Chem. Comm.*, 1956, **21**, 79.
[8] Hörhammer and Müller, *Arch. Pharm.*, 1954, **287**, 310.

diazomethane could not be achieved by the method of Marini-Bettolo *et al.*[9] or Flores and Herran,[10] but use of dimethyl sulphate gave a product that was separated by chromatography into unchanged casticin and artemetin. Thus casticin is 5,3′-dihydroxy-3,6,7,4′-tetramethoxyflavone. This has not previously been found in Nature.

Experimental

M. p.s were taken on a Kofler hot stage unless otherwise stated. Ultraviolet absorption spectra were determined for 95% ethanol solutions with a Unicam spectrophotometer, model S.P. 500.

Extraction.—The seeds of *Vitex agnus castus* were collected on the Isle of Lošinj, Yugoslavia, during October. The ground seeds were extracted (Soxhlet) with light petroleum (b. p. 30—50°) for 26 hr. and the deposited yellow substance, amounting to 0·1% of the weight of seeds, was filtered off. It crystallised from benzene–light petroleum as prisms, m. p. 186—187°, $\lambda_{max.}$ 258, 350, and 272 infl. mμ (log ε 4·32, 2·37, and 4·06, respectively), $\lambda_{max.}$ in alkaline solution 276 and 380 mμ (log ε 4·70 and 4·14, respectively), $\lambda_{max.}$ (mull) 2·87, 3·47, 6·02, 6·22, 6·28, 6·42, 6·58, 6·69, 6·94, 7·30, 7·80, 7·89, 8·06, 8·19, 8·59, 8·79, 8·93, 9·12, 10·04, 10·29, 11·52, 12·65, 13·04, and 13·71 μ (Found: C, 60·7; H, 4·9; O, 34·3; OMe, 32·1. $C_{19}H_{18}O_8$ requires C, 61·0; H, 4·85; O, 34·2; 4OMe, 33·2%). *Casticin* is insoluble in water and cold light petroleum, slightly soluble in hot light petroleum, and readily soluble in acetone, benzene, ethanol, and chloroform. It gives an olive-green colour with ferric chloride solution and a bright yellow precipitate with lead acetate. Reduction with magnesium and hydrochloric acid or with zinc and hydrochloric acid gave a deep crimson colour. In sodium hydroxide or concentrated sulphuric acid it gave an intense yellow colour. The Wilson reaction with an acetone solution of boric and citric acid was positive. Casticin has R_F 0·26 in formamide–benzene–decalin (mobile phase benzene–decalin, 1 : 1, saturated with formamide), appearing in ultraviolet light as a dark brown spot, that gives an orange-red colour when sprayed with diazotised sulphanilic acid.

Casticin (50 mg.) was treated [11] with acetic anhydride (0·5 ml.) and a drop of 60% perchloric acid at room temperature. The *diacetate* (45 mg.), isolated after ½ hr., crystallised from ethanol and then ethyl acetate as colourless needles, m. p. 178—179° (capillary), $\lambda_{max.}$ 276, 335 mμ (log ε 4·18, 4·26) (Found: C, 60·1; H, 4·7; O, 34·9. $C_{23}H_{22}O_{10}$ requires C, 60·3; H, 4·8; O, 34·9%), giving no colour with ferric chloride solution.

Casticin (500 mg.) was refluxed with anhydrous potassium carbonate (5·5 g.) and diethyl sulphate (2 ml.) in acetone (70 ml.) for 48 hr. Evaporation yielded a residue of *diethyl ether* which crystallised from methanol and then light petroleum as colourless needles (450 mg.), m. p. 115—116°, $\lambda_{max.}$ 240, 330 mμ (log ε 3·15, 3·20) (Found: C, 64·0; H, 6·1; O, 29·7. $C_{23}H_{26}O_{10}$ requires C, 64·2; H, 6·1; O, 29·75%), giving a negative ferric chloride reaction.

Methylation of Casticin.—A solution of casticin (100 mg.) in dry acetone (15 ml.) was heated under reflux with anhydrous potassium carbonate (1 g.) and dimethyl sulphate (0·4 ml.) for 6 hr. Colourless needles, m. p. 141—142° (Found: C, 62·5; H, 5·7. Calc. for $C_{21}H_{22}O_8$: C, 62·7; H, 5·5%), were obtained. The m. p. was not depressed on admixture with authentic hexa-*O*-methylquercetagetin. The ultraviolet spectra [$\lambda_{max.}$ 242, 335 mμ (log ε 4·49, 4·54)] and R_F values (dimethylformamide–decalin and formamide–benzene) of the samples were identical.

Demethylation of Casticin.—Casticin (253 mg.) was heated under reflux for 1 hr. with hydriodic acid (6 ml.; *d* 1·7) and acetic anhydride (15 ml.). The yellow precipitate (164 mg.) formed when the mixture was poured into saturated aqueous sodium dithionite, crystallised from ethanol in yellow needles, m. p. >300° (decomp.). The ultraviolet spectrum [$\lambda_{max.}$ 259, 361, and 272 infl. mμ (log ε 4·23, 4·34, and 4·15 respectively)] was identical with that of quercetagetin.[6] The demethylated product (162 mg.), when acetylated as above, gave the hexa-acetate, prisms (from ethyl acetate), m. p. and mixed m. p. 210—211°. The R_F values (formamide–benzene) of the samples were identical.

Hydrolysis of Casticin.—Casticin (100 mg.) was heated under reflux for 4 hr. under nitrogen with ethanolic 20% potassium hydroxide solution (10 ml.). The solvent was then removed

[9] Marini-Bettolo and Chiavarelli, 16th Internat. Congr. Pure Appl. Chem., Paris, 1957, Résumés II, p. 212.
[10] Flores and Herran, *Tetrahedron*, 1958, **2**, 308.
[11] Cf. Briggs and Locker, *J.*, 1949, 2157.

under reduced pressure and the residue dissolved in water. The solution was saturated with carbon dioxide and extracted with ether. Material obtained from the dried extract (43 mg). recrystallised from ethanol as yellow needles, m. p. 129—130° (Baker, Nodzu, and Robinson [12] give m. p. 129—130° for 2,6-dihydroxy-ω,4,5-trimethoxyacetophenone). The aqueous solution was acidified with sulphuric acid and extracted with ether. The acid (41 mg.) crystallised from ethanol (charcoal) as needles, m. p. 248° (capillary) (Found: OMe, 18·8. Calc. for $C_8H_8O_4$: 1OMe, 18·5%), undepressed in m. p. on admixture with isovanillic acid,[13] with which it was identical in R_F (propan-2-ol–ammonia) and colours of spots. A depression of the m. p. to 224° was observed on its admixture with vanillic acid.

Hydrolysis of Casticin Diethyl Ether.—The ether (385 mg.) was heated under reflux for 10 hr. with ethanolic 20% potassium hydroxide solution (20 ml.). The acidic fraction (219 mg.) was crystallised successively from aqueous ethanol (charcoal) and benzene, forming colourless needles, m. p. 164° (capillary) (Späth and Bernhauer [14] report 3-ethoxy-4-methoxybenzoic acid, m. p. 165—166°).

Partial Methylation of Casticin.—Casticin (570 mg.) was refluxed with anhydrous potassium carbonate (2·5 g.) and dimethyl sulphate (0·06 ml.) in dry acetone (50 ml.) for 2 hr. The solvent was evaporated under reduced pressure. The residue was chromatographed on a column of cellulose powder (Whatman Standard Grade; 200 g.) and formamide (66 g.). Benzene–light petroleum (b. p. 30—40°) (4 : 1) gave first several fractions showing only one spot on paper chromatograms; these were combined and the solvents were evaporated; the residue crystallised from benzene–light petroleum in yellow needles (235 mg.), m. p. 160—161·5° undepressed on admixture with artemetin, and identical with it in ultraviolet spectrum and R_F values (formamide–benzene and dimethylformamide–decalin).

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[12] Baker, Nodzu, and Robinson, *J.*, 1929, 81.
[13] Perkin and Stoyle, *J.*, 1923, **123**, 3171.
[14] Späth and Bernhauer, *Ber.*, 1925, **58**, 203.