Der Dextranträger führt also zu keinen messbaren Veränderungen der untersuchten kinetischen Eigenschaften, was mit den bisherigen Vorstellungen über die Beeinflussung des aktiven Zentrums durch das Mikromilieu des Trägers übereinstimmt[6]. Danach sind aufgrund von Diffusionsbehinderung insbesondere für makromolekulare Substrate höhere K_m-Werte und in solchen Fällen Änderungen in der pH-Aktivitätskurve sowie den K_m-Werten zu erwarten, wenn sowohl die Substrate als auch der Träger ionisierte Gruppen enthalten. Für Sephadex ist der Gehalt an dissoziierenden Gruppen äusserst gering. Offenbar werden bei der Aktivierung des Trägers durch Bromcyan auch nur wenige ionisierende Gruppen eingeführt, die zu keinen Änderungen der kinetischen Parameter führen.

Summary. Yeast hexokinase was coupled to Sephadex by means of cyanogen bromide. The activity of the fixed enzyme was 2% of that before coupling. The activity of the noncoupled part of the enzyme was also decreased to the same degree. This decrease cannot be caused by the coupling reaction itself. The Michaelis-constants and the pH-activity curve of the coupled enzyme do not differ significantly from that of the free enzyme.

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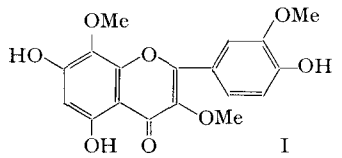
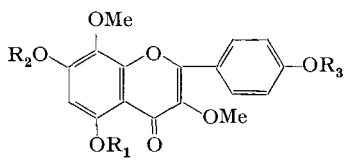
The Syntheses of 3,8-Dimethoxy-5,7,4′-trihydroxyflavone and Related Compounds

Recently, 2 new flavonoid pigments were isolated from *Cyanoestegia angustifolia* Turcz. (Verbenaceae) by E. L. GHISALBERTI et al.[1]. These authors proposed the structures of these pigments as 5,7,4′-trihydroxy-3,8,3′-trimethoxyflavone (I) and 3,8-dimethoxy-5,7,4′-trihydroxyflavone (II) on the basis of spectroscopic and degradative studies. In previous papers[2], we reported the synthesis of I and its identity with a natural pigment. Now we describe the total synthesis of II by 2 different methods.

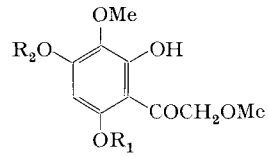
According to Allan-Robinson's flavone synthesis, the condensation of 2,4-dihydroxy-3,6,ω-trimethoxyacetophenone (III)[3] with 4-benzyloxybenzoic anhydride (IV) gave 4′-benzyloxy-7-hydroxy-3,5,8-trimethoxyflavone (V, m.p. 240–242°, Found: C, 68.85; H, 4.97. $C_{25}H_{22}O_7$ requires: C, 69.11; H, 5.10%), which was converted into its benzyl ether (VI, m.p. 149–150°, Found: C, 73.12; H, 5.39. $C_{32}H_{28}O_7$ requires: C, 73.27; H, 5.38%). VI was also obtained from 4-benzyloxy-2-hydroxy-3,6,ω-trimethoxyacetophenone (VII)[3] and IV. The oxidation of VI with nitric acid, followed by the subsequent reduction with sodium sulfite yielded 7,4′-dibenzyloxy-5,8-dihydroxy-3-methoxyflavone (VIII, m.p. 201–203°, Found: C, 72.39; H, 4.68. $C_{30}H_{24}O_7$ requires: C, 72.57; H, 4.87%). Partial methylation of VIII with diazomethane gave 7,4′-dibenzyloxy-3,8-dimethoxy-5-hydroxyflavone (IX, m.p. 161–162°, Found: C, 73.19; H, 5.11. $C_{31}H_{26}O_7$ requires: C, 72.93; H, 5.13%). Debenzylation of IX with hydrogen yielded the expected flavone II (m.p. 247–249° and 257–258°, UV λ_{max}^{EtOH} nm (log ε): 274 (4.36), 310$_i$ (4.16)[4], 326 (4.19), 360 (4.15). Found: C, 61.82; H, 4.39. $C_{17}H_{14}O_7$ requires: C, 61.82; H, 4.27%) (natural one[5],



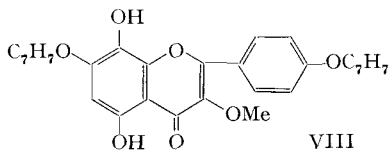
I



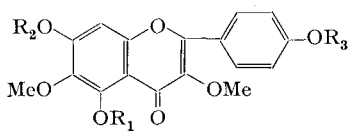
II $R_1 = R_2 = R_3 = H$
V $R_1 = Me$, $R_2 = H$, $R_3 = C_7H_7$
VI $R_1 = Me$, $R_2 = R_3 = C_7H_7$
IX $R_1 = H$, $R_2 = R_3 = C_7H_7$
X $R_1 = R_2 = R_3 = CH_3CO$
XIII $R_1 = R_2 = H$, $R_3 = C_7H_7$



III $R_1 = Me$, $R_2 = H$
VII $R_1 = Me$, $R_2 = C_7H_7$
XI $R_1 = R_2 = H$



VIII



XII $R_1 = H$, $R_2 = CH_3CO$, $R_3 = C_7H_7$
XIV $R_1 = R_2 = R_3 = H$
XV $R_1 = R_2 = R_3 = CH_3CO$

[1] E. L. GHISALBERTI, P. R. JEFFERIES and C. I. STACEY, Aust. J. Chem. *20*, 1049 (1967).
[2] K. FUKUI, M. NAKAYAMA and T. HORIE, Experientia *24*, 417 (1968); K. FUKUI, T. MATSUMOTO, M. NAKAYAMA and T. HORIE, Bull. chem. Soc. Japan *41*, 2805 (1968).
[3] V. D. N. SASTRI and T. R. SESHADRI, Proc. Indian Acad. Sci. *24* A, 238 (1946).
[4] *i*, inflection point.
[5] The natural pigment, kindly supplied by Prof. P. R. JEFFERIES, the University of Western Australia, was measured in this laboratory.

m.p. 246–248° and 256–258°, UV λ_{max}^{EtOH} nm (log ε): 274 (4.28), 310$_i$ (4.08)[4], 326 (4.13), 360 (4.09)) (lit.[1] m.p. 242–244° and 254–256°) (the triacetate (X): m.p. 170–171°; lit.[1] m.p. 167–168°), whose identity with the natural product was confirmed by mixed m.p. determination and UV- and IR-spectral comparison.

II was also obtained from 3,ω-dimethoxy-2,4,6-trihydroxyacetophenone (XI) in a manner similar to that described earlier[2,6]. Condensation of XI with IV gave a mixture of flavones. After partial acetylation[7], the reaction mixture was purified by recrystallization from ethyl acetate to give 7-acetoxy-4′-benzyloxy-3,6-dimethoxy-5-hydroxyflavone (XII, m.p. 209–210°, Found: C, 67.47; H, 4.86. $C_{26}H_{22}O_8$ requires: C, 67.52; H, 4.80%). The residue of the mother liquor was hydrolyzed with alkali to give 4′-benzyloxy-5,7-dihydroxy-3,8-dimethoxyflavone (XIII, m.p. 193–194°, UV λ_{max}^{EtOH} nm (log ε): 275.5 (4.39), 320 (4.25). Found: C, 68.54; H, 4.68. $C_{24}H_{20}O_7$ requires: C, 68.56; H, 4.80%). Debenzylation of XIII afforded II, which was identical with the sample described above.

Treatment of XII with alkali, followed by debenzylation, gave 3,6-dimethoxy-5,7,4′-trihydroxyflavone, an isomer of II,(XIV, m.p. 227–228°, UV λ_{max}^{EtOH} nm (log ε): 271 (4.16), 340 (4.22). Found: C, 61.93; H, 4.21. $C_{17}H_{14}O_7$ requires: C, 61.82; H, 4.27%) (the triacetate (XV): m.p. 170–172°). It has been observed[6,8] that in the NMR-spectra of the aromatic proton of the ring A in flavonoid, a signal of C_6-H appeared at upper field than that of C_8-H. Due to this fact, an aromatic proton signal of XV appearing at 7.31 (singlet) corresponds to C_8-H and a signal of X at 6.78 corresponds to C_6-H[9].

Zusammenfassung. 3,8-Dimethoxy-5,7,4′-trihydroxyflavon und 3,6-Dimethoxy-5,7,4′-trihydroxyflavon wurden synthetisiert.

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16 December 1968.

[6] K. Fukui, T. Matsumoto, S. Nakamura, M. Nakayama and T. Horie, Experientia *24*, 108 (1968); Bull. chem. Soc. Japan *41*, 1413 (1968). – K. Fukui, M. Nakayama and T. Horie, Experientia *24*, 769 (1968). – T. Horie, Experientia *24*, 880 (1968).
[7] M. Simokoriyama, Bull. chem. Soc. Japan *16*, 284 (1941).
[8] C. A. Henrick and P. R. Jefferies, Aust. J. Chem. *17*, 934 (1964).
[9] The NMR-spectra were measured with Hitachi R-20 NMR-spectrometer, using tetramethylsilane as internal standard (δ-value in $CDCl_3$).

The Structure of WI3, the First Optically Active Biflavone of the Amentoflavone Series

We have previously reported the isolation from *Araucaria cookii* of the first optically active naturally occurring biflavones, these being members of the cupressuflavone series[1]. From the same source we have isolated a new substance WI3, m.p. 273°, $C_{34}H_{26}O_{10}$ (mol. wt. 594.151227), $[\alpha]_D^{34°}$ (pyridine-ethanol) +41° λ_{max} (ethanl) 274,334 nm, diacetate (mol. wt. 678.176231), m.p. 220–225°, $C_{38}H_{30}O_{12}$ and a dimethyl ether m.p. 217–218°, $C_{36}H_{30}O_{10}$ (mol. wt. 622.182339).

The UV-spectra of these compounds and the usual colour tests indicated that the molecules were flavonoid in nature. The mass spectrum of the dimethyl ether showed a large peak at *m/e* 311, indicating that each flavonoid unit had 3 methoxy groups.

The NMR-spectrum of the substances made it clear that WI3 and its derivatives are not members of the cupressuflavone series[1]. The dimethyl ether was investigated in detail, using double irradiation techniques, and it was possible to assign a τ value to every proton, with the exception of H-3 and H-3″ which could not be distinguished (see Table). In particular, associated with rings B and E, there is evidenced an A_2B_2 system and also an ABX system. Thus a ring B of a flavone unit is associated with a linkage to ring A of another such unit. In particular the τ values show that C-3′ is linked to C-6″ or C-8″.

By analogy with cupressuflavone hexamethyl ether, the single methoxy group showing below τ 6.0 is assigned to the 5″-OMe, attached to an 8-linked flavone unit, whilst the protons on ring A are assigned by analogy with 5,7-dimethoxyflavone[2]. All methoxy groups were moved upfield on change of solvent from chloroform to benzene, as with cupressuflavone hexamethyl ether[1], showing that every methoxy group has a least one *ortho*-proton, and therefore a C-8 rather than a C-6 linkage is indicated. An authentic sample of (±)-amentoflavone hexamethyl ether was obtained[3] and shown to give an NMR-spectrum identical with WI3 dimethyl ether in all respects including solvent dependent methoxy shifts. The IR-, UV- and mass spectrum were also identical.

WI3 diacetate has 3 singlets, 2 like those in the dimethyl ether at τ 3.50 and τ 3.44, but the other had moved downfield to τ 3.27, a shift of the order to be expected of H-6 on acetylation of a 5-hydroxy group of

Proton	Methyl WI3	Acetyl WI3	WI3
H-2′	2.10q $J_1 = 8$ c/s $J_2 = 3$ c/s	2.08q $J_1 = 9$ c/s $J_2 = 3$ c/s	2.08q
H-3′	2.88d $J = 8$ c/s	2.86d $J = 9$ c/s	2.89d $J = 9$ c/s
H-6′	2.16d $J = \sim 3$ c/s	2.13d $J = \sim 3$ c/s	2.16d
H-2‴ (6‴)	2.62d $J = \sim 9$ c/s	2.64d $J = 9$ c/s	2.56d $J = 9$ c/s
H-3‴ (5‴)	3.24d $J = \sim 9$ c/s	3.23d $J = 9$ c/s	3.21d $J = 9$ c/s
H-3	[3.48s	[3.50	[3.50
H-3″	3.42s]	3.44]	3.44
H-6″	3.38s	3.27	3.42]
H-6	3.66d $J = 3$ c/s	3.42d $J = 3$ c/s	3.68d $J = 3$ c/s
H-8	3.52d $J = 3$ c/s	3.19d $J = \sim 3$ c/s	3.60d $J = 3$ c/s
OMe	5.94, 6.08 6.12, 6.18, 6.25 6.27	6.16 (6H) 6.23, 6.25	6.17, 6.19 6.22, 6.24
OAc	–	7.52, 7.59	–
OH	–	–	−2.56, −3.07

Figures given in τ values. Methyl WI3 and Acetyl WI3 run in $CDCl_3$, WI3 in $(CD_3)_2CO$ with Me_4Si as internal standard.