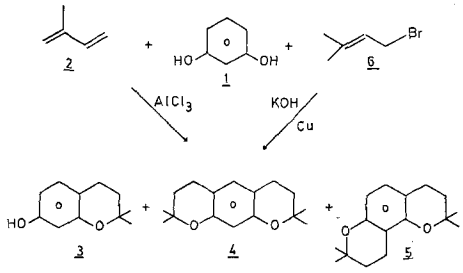
Reaction of Resorcinol with Isoprene

In connection with our interest in the synthesis of *O*-heterocyclic natural products, we investigated the reaction of resorcinol with isoprene.

The condensation of resorcinol (**1**) with isoprene (**2**) in carbon disulfide and aluminium chloride afforded a brown oil. Fractional distillation gave two easily separable fractions. The first fraction, b.p. 140–145 °C/0.2 mm Hg consisted of pure 7-hydroxy-2, 2-dimethylchroman (**3**) as identified by IR and NMR. Compound **3** was previously prepared by Clemmensen reduction of 7-hydroxy-2, 2-dimethylchroman-4-one[1] and by the condensation of resorcinol with 2-methyl-3-butene-2-ol in aqueous citric acid solution[2].



The mass spectrum of **3** showed besides strong M^+ and M^+-15, a base peak at *m/e* 123 resulting from a retro-Diels-Alder fragmentation accompanied by a hydrogen shift. Formation of the phenol **3** can be explained by the acid-catalysed cyclization of the intermediate 4-γ, γ-dimethylallylresorcinol.

The second fraction, b.p. 160–172 °C/0.2 mm Hg was a mixture of 6, 7-dihydro-2, 2, 8, 8-tetramethyl-8H-[1]pyrano [3, 2-g] chroman (**4**) and 9, 10-dihydro-2, 2, 8, 8-tetramethyl-8H- [1] pyrano [2, 3-h] chroman (**5**), separated by GLC (SE-30). The chromans **4** and **5** were derived from the phenol **3** by further isoprenylation and cyclization.

A distinction between the linear **4** and the angular **5** dichromans could be made by NMR. The symmetric compound **4** showed one signal for both gem-dimethyl groups and the aromatic protons appeared as two singlets. Compound **5** is asymmetric and exhibited different peaks for the gem-dimethyl groups, wihle the aromatic protons displayed a AB pattern.

Compound **4**: ν_{max} (NaCl) 1625, 1580, 1490 cm^{-1} (aromatic); δ (CCl_4): 1.28 (12H, s, $4\times CH_3$); 1.70 (4H, t, J 7.0 cps, $2\times CH_2$); 2.62 (4H, t, J 7.0 cps, $2\times CH_2$); 6.01 (1H, s, Ar-H); 6.52 (1H, s, Ar-H). Compound **5**: ν_{max} (NaCl) 1615, 1590 and 1485 cm^{-1} (aromatic); δ (CCl_4) 1.29 (6H, s, $2\times CH_3$); 1.31 (6H, s, $2\times CH_3$); 1.69 (2H, t, J 6.2 cps, CH_2); 1.73 (2H, t, J 6.0 cps, CH_2); 2.53 (2H, t, J 6.2 cps, CH_2); 2.64 (2H, t, J 6.0 cps, CH_2); 6.14 (1H, d, J 8.3 cps, Ar-H); 6.62 (1H, d, J 8.3 cps, Ar-H). The mass-spectrum of **5** exhibited simple breakdown patterns. A retro-Diels-Alder fragmentation with a hydrogen transfer occured to give the base peak (*m/e* 191). The much less abundant fragment ion at *m/e* 190 may originate from a retro-Diels-Alder reaction, followed by expulsion of a methyl radical (*m/e* 175) and a retro-Diels-Alder fragmentation with a hydrogen shift (*m/e* 135).

The same compounds **3**, **4** and **5** were obtained by the reaction of resorcinol (**1**) with 1-bromo-3-methyl-2-butene (**6**) in aqueous potassium hydroxide solution and copper powder.

Zusammenfassung. Die Synthese von 7-Hydroxy-2, 2-dimethylchroman, 6, 7-Dihydro-2, 2, 8, 8-tetramethyl-8H-[1]pyrano[3, 2-g]chroman und 9, 10-Dihydro-2, 2, 8, 8-tetramethyl-8H-[1]pyrano[2, 3-h]chroman aus Resorcin und Isopren oder 1-Bromo-3-methyl-2-buten wird beschrieben.

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State University of Ghent, Faculty of Agricultural Sciences, Laboratory of Organic Chemistry, B–9000 Ghent (Belgium), 8 January 1973.

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[2] R. J. MOLYNEUX, L. JURD, Tetrahedron *26*, 4743 (1971).

Robustaflavone – the First Member of a New Series of Biflavones

Naturally occurring flavanoids with the 6- and 8-positions carbon-carbon linked to a variety of substituents, viz. methyl, $\gamma\gamma$-dimethallyl and glucosyl, are well known and constitute isomeric pairs. Surprisingly enough the interflavonyl linkage of biflavones is found to implicate only position -8 out of the above 2, except in naturally occurring hinokiflavone (Ia). The discoveries of cupressuflavone[1] (II) and agathisflavone[2,3] (IIIa) provided the first example of such a pair of naturally occurring isomers. In the present communication we wish to report the isolation and characterization of a new biflavone (IVa) as hexamethyl ether (IVb) which with amentoflavone (Va) constitutes the second example of such an isomeric pair of naturally occuring biflavones. The new biflavone (IVa) isolated from the leaf extract of *Agathis robusta* has been named as 'robustaflavone'. A monomethyl ether of IVa is also indicated (TLC).

The leaf extract of *Agathis robusta*, by the usual methods of purification, gave a biflavone mixture which separated by preparative layer chromatography (BPF)[4] into six fractions labelled as AgI-AgVI. They were found to be chromatographically (TLC) identical with the 6 bands already reported in *A. palmerstonii*[3] and *A. alba*[3,5]. The fractions AgII and AgIV, although reported as consisting of single entities from the previously reported plants[2,3,5], were found after methylation to be mixtures in the present case. Rf value considerations and characteristic fluorescence in UV-light[4] indicated each of them as mixtures of complete methyl ethers of agathisflavone (major) and a

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[3] NIZAM U. KHAN, W. H. ANSARI, J. N. USMANI, M. ILYAS and W. RAHMAN, Phytochemistry *10*, 2129 (1971).
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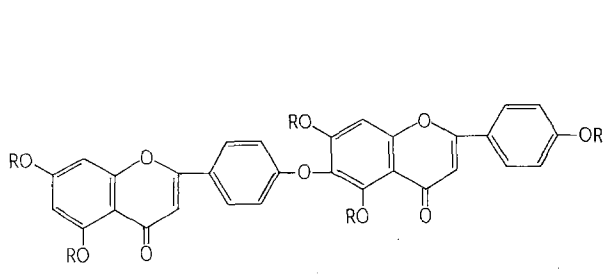
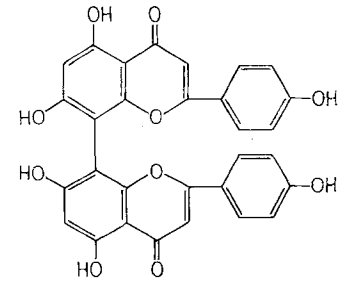
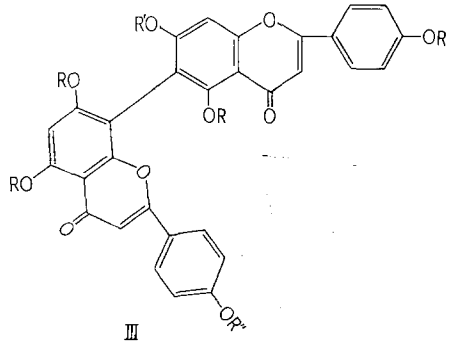


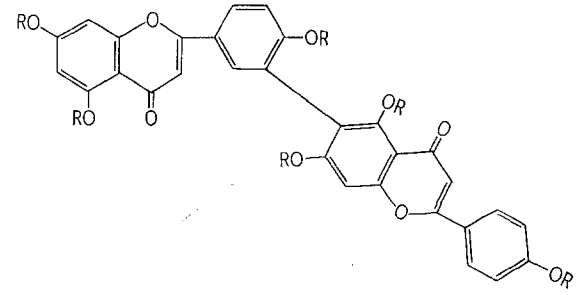
I
(a) R=H, (b) R=Me



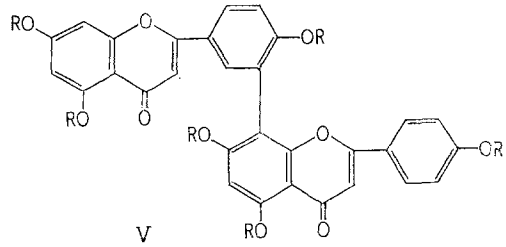
II



III
(a) R=R′=R″=H
(b) R=R′=R″=Me
(c) R=R″=H; R′=Me
(d) R=H; R′=R″=Me



IV
(a) R=H
(b) R=Me



V
(a) R=H
(b) R=Me

The NMR spectral data of Hexa-*O*-methyl robustaflavone in $CDCl_3$

Protons	Chemical shifts (δ)	S-values by Eu(FOD)$_3$
OCH_3-5,5″	3.93, 3.61	10.58, 2.64
7,7″	(3.88, 3.82)	(0.74, 0.56)
4′4‴	3.86, 3.88	0.32,– 0.06
H-3,3″	6.65[a] (2H, s)	(0.30[a], 0.16)
6	6.35 d	4.84
8,8″	6.58 d, 6.88 s[a]	1.20, 0.50[a]
2′, 6′	7.81 d, 7.87 q	1.42, 0.36
2‴, 6‴	7.87 (2H, d)	−0.08
5′	7.09 d	0.52
3‴, 5‴	7.02 (2H, d)	−0.06

[a] Alternative assignment is possible for these figures as well as for those in parentheses. Values are represented in ppm from internal TMS. S-values (ppm) are represented as the slopes of straight lines obtained by plotting the shift values induced by Eu(FOD)$_3$ for every proton signal against the molar ratio of Eu(FOD)$_3$.

hitherto unreported naturally occurring biflavone 'robustaflavone' (minor). Counter current distribution of AgII and AgIV gave only major components which were identified by NMR as 7-*O*-methylagathisflavone (IIIc)[2,5] and 7,4‴-di-*O*-methylagathisflavone (IIId)[2,5] respectively. Each mixture was, therefore, methylated and subjected to preparative layer chromatography to give hexa-*O*-methylagathisflavone (IIIb) and hexa-*O*-methyl robustaflavone (IVb). The compound (IVb), $C_{36}H_{30}O_{10}$ (M^+, 622.1791) melted at 305–308 °C. NMR spectral data of IVb taken in $CDCl_3$ are given in the Table.

The mode of interflavonyl linkage as C3′-C6″ in robustaflavone (IVa) was established from benzene-induced solvent shift studies of methoxy resonances of IVb. On change of solvent from $CDCl_3$ to C_6D_6, a methoxy group at 3.61 moved downfield, whereas the other 5 showed upfield shifts like penta-*O*-methyl hinokiflavone[6] (Ib) and hexa-*O*-methylagathisflavone[2] (IIIb). This finding is compatible with the structure of robustaflavone (IVa)

[6] A. Pelter, R. Warren, J. N. Usmani, M. Ilyas and W. Rahman, Tetrahedron Lett. *49*, 4259 (1969).

because OCH_3-5'' in this case is located in similar environments as OCH_3-5 of IIIb and OCH-5'' of Ib.

The above mode of linkage gains further support from mass spectral studies of IVb. The presence of *m/e* 311 as a major peak in IVb and IIIb, but very minor one in Vb, is of considerable significance. This may be attributed to facile carbon-carbon cleavage in IVb and IIIb due to steric reasons.

Lanthanide-induced shift studies by $Eu(FOD)_3$ have also been carried out to evaluate proton chemical shifts of IVb. S-values of every proton are listed in the Table. We have recently reported[7] that H-6 of flavone nucleus on addition of $Eu(FOD)_3$ shows a considerable downfield shift (2.76 $\sim$ 5.80 ppm in S-values) in comparison with H-8 or H-3 (less than 1.14 ppm) while the side phenyl protons are shifted to a very small extent. A singlet at 6.88 ppm assigned to H-8'' gave a small S-value (0.50 ppm). A large S-value of OCH_3-5 (10.58 ppm) would mean that complexation occurs mostly at this side of the molecule. The S-value of H-2' (1.42 ppm) is larger than usual ($-0.50 \sim 0.56$ ppm)[7] perhaps because the side phenyl group (at C-3') is attached to 6-position of the other flavone nucleus. These observations are in accord with the previous findings and are compatible with the structure of robustaflavone (IVa). Further, paramagnetic induced shift studies disentangled the signals of H-2' (7.81 d) and H-6' (7.87 q) which were found overlapping with a doublet of H-2''', 6''' (7.87 d).

The synthetic sample of IVb was obtained in a yield of 10 to 15% through Wessely-Moser rearrangement of hexa-*O*-methylamentoflavone (Vb) followed by methylation. Both the samples had the same m.p. 305–308° and showed no depression on admixture. Rf values, fluorescence in UV-light, UV, IR and NMR spectral data of the 2 samples were also in good accordance. Judging from the Rf value (TLC) it was deduced that robustaflavone was present in AgII and its monomethyl ether in AgIV.

Zusammenfassung. Isolierung und Strukturaufklärung eines neuen Typs der Biflavone aus *Agathis robusta*.

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30 November 1972.

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[8] Acknowledgment: The author is thankful to Council of Scientific and Industrial Research (CSIR), New Delhi, India, for financial assistance.

The Synthesis of a Decapeptide with Glycosidase Activity[1]

Copolymers of Glu[2] and hydrophobic amino acids have been shown to have substantial lysozyme-like[3,4] and non-specific glycosidase activities[5]. These copolymers were synthesized as they would have unionized and ionized carboxyl functions in their hydrophobic and hydrophilic regions respectively. Contact with a polysaccharide substrate was expected to lead to protonation of proximally placed glycosidic oxygen atoms by unionized carboxyl functions. Bond cleavage would then occur if the resulting carbonium ion could be stabilized by a suitably placed carboxylate anion.

The extension of this concept is the synthesis of a small peptide with suitably disposed carboxyl functions of which one should be in a hydrophobic environment and at least one in a hydrophilic environment. The design of such a peptide requires anticipation of its conformation. In the absence of adequate information on the conformation of peptide sequences in solution, we projected our synthesis to achieving conformational control in the solid state.

In an analysis of amino acid sequence whose three-dimensional structures have been determined by X-ray crystallography, KOTELCHUCK and SCHERAGA[6] have identified the α-helical and non-helical character of nearly 80% of the individual peptides in protein molecules. According to the rules formulated, the initiation of an α-helix requires 4 helix-making amino acids in a row so that the helix grows towards the C-terminal end unless interrupted by 2 helix-breaking amino acids in succession. If these characteristics are also manifest in smaller peptide sequences, the decapeptide Glu-Phe-Ala-Ala-Glu-Glu-Ala-Ala-Ser-Phe (I) might be expected to have a tendency to form an α-helix as the only helix-breaking amino acid in the sequence is Ser-9[6]. Further, if this peptide were to adopt an α-helix conformation, Glu-6 would have its carboxyl function in a hydrophobic environment as it would be flanked above and below by the benzene rings of Phe-2 and Phe-10 (Figure 1) and the adjacent Ala-7 methyl would also contribute to its hydrophobic environment. Glu-5, on the other hand, would be in a hydrophilic environment and the carboxyl of this amino acid residue or of Glu-1 with the carboxyl of Glu-6 could provide the catalytic site of this enzyme model.

Decapeptide I was synthesized conventionally[7]. Z-Ser(Bu^t) was condensed with Phe-OBu^t in the presence of DCC to give Z-Ser(Bu^t)-Phe-OBu^t (II), crystallized from C_6H_{14}, mp 87–88°, $(\alpha)_D^a$ + 6.1°, yield 88%. II was treated with H_2/Pd black and the product condensed with Z-Ala-ONSu to obtain tripeptide Z-Ala-Ser(Bu^t)-Phe-OBu^t (III), crystallized from CH_2Cl_2-C_6H_{14}, mp 114–115°, $(\alpha)_D^a$ −19.7°, yield 76%.

Z-Glu(OBu^t) was condensed with Ala-OMe with the aid of DCC. The product Z-Glu(OBu^t)-Ala-OMe crystallized from CH_2Cl_2-C_6H_{14}, mp 105–106°, $(\alpha)_D^a$ −49.7°, yield 85%, was treated with H_2/Pd black to yield Glu(OBu^t)-

[1] Communication No. 1722 from the Central Drug Research Institute constitutes synthetic substitute enzymes Pt. V, presented in part at the I.U.P.A.C. symposium on Natural Products, New Delhi, Feb. 1972.

[2] Abbreviations in accordance with IUPAC-IUB Commission on Biochemical Nomenclature, Biochemistry *11*, 1726 (1972).

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[7] Satisfactory C, H and N, analysis obtained for all peptides synthesized. $(\alpha)_D^a$ and $(\alpha)_D^b$ are reported for 1% solutions in MeOH at 25° and in DMF at 34° respectively.