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*.

A. K. VARSHNEY[8], W. RAHMAN, M. OKIGAWA and N. KAWANO

Department of Chemistry, Aligarh Muslim University, Aligarh (India) and Faculty of Pharmaceutical Sciences, Nagasaki University, Nagasaki (Japan),
30 November 1972.

[7] M. OKIGAWA, N. KAWANO, W. RAHMAN and M. M. DHAR, Tetrahedron Lett. *40*, 4125 (1972).

[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).

[3] V. K. NAITHANI and M. M. DHAR, Biochem. biophys. Res. Commun. *29*, 368 (1967).

[4] S. SRIVASTAVA, K. B. MATHUR and M. M. DHAR, Experientia *26*, 11 (1970).

[5] K. B. MATHUR, P. K. CHAKRAVARTY, S. SRIVASTAVA and M. M. DHAR, Indian J. Biochem. Biophys. *8*, 90 (1971).

[6] D. KOTELCHUCK and H. A. SCHERAGA, Proc. natn. Acad. Sci. USA *62*, 14 (1969).

[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.

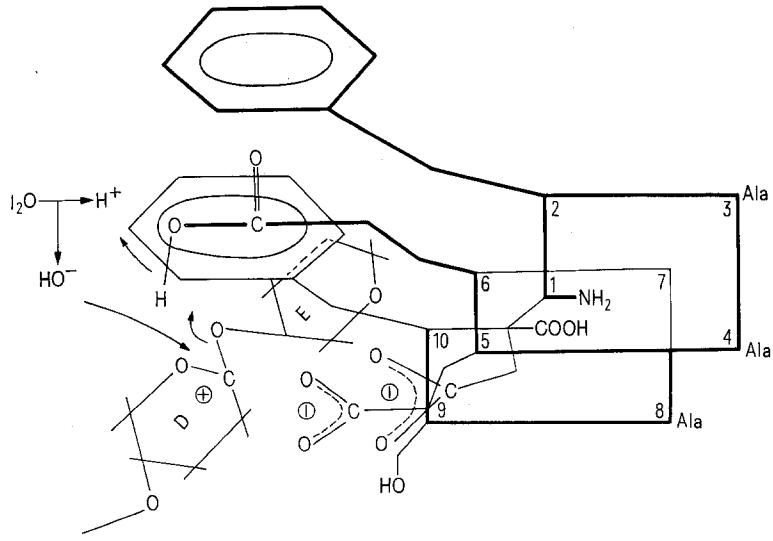


Fig. 1. Decapeptide enzyme model.

Ala-OMe (IV). Condensation of IV with Z-Glu(OBu^t)-ONSu yielded Z-Glu(OBu^t)-Glu(OBu^t)-Ala-OMe (V), crystallized from CH_2Cl_2-C_6H_{14}, mp 117–118°, $(\alpha)_D^a$ −54.5°, yield 69%. V was treated with $NH_2.NH_2.H_2O$ (98%) to yield Z-Glu(OBu^t)-Glu(OBu^t)-Ala-N_2H_3 (VI), crystallized from MeOH-Et_2O, mp 159–160°, $(\alpha)_D^b$ −5°, yield 70%. III was decarbobenzoxylated with H_2/Pd black and condensed with the azide from VI to yield the hexapeptide Z-Glu(OBu^t)-Glu(OBu^t)-Ala-Ala-Ser(Bu^t)-Phe-OBu^t (VII), crystallized from EtOAc, mp 226–227°, $(\alpha)_D^b$ −6.3°, yield 60%.

Incorporation of ^3H in I. HBr

Tritiation time (h)	Counts (mg/5 min)
0.5	1985
0.5[a]	4769
0.75	1965
1	2060
3	2169
6	2225
12	2220
24	2223

[a] Tritiation carried out in presence of TFA

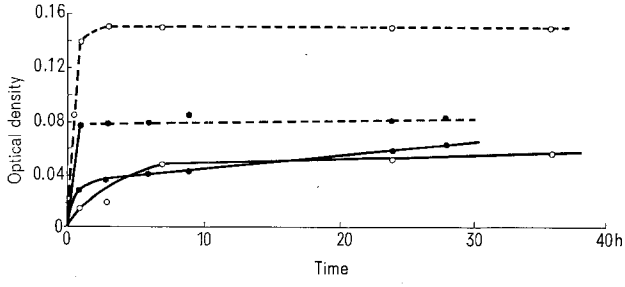


Fig. 2. Hydrolysis of chitin by hen egg-white lysozyme (0.5 mg/ml) (---) and decapeptide I (2.5 mg/ml) (—) at pH 5.8 (●) and 7.0 (○).

Z-Ala-Ala-OMe[8] was decarbobenzoxylated with HBr/AcOH and the free base condensed with Z-Phe-ONSu to give Z-Phe-Ala-Ala-OMe (VIII), crystallized from EtOAc, mp 192–193°, $(\alpha)_D^b$ −23.8°, yield 82%. VIII in turn was treated with HBr/AcOH and the free base condensed with Z-Glu(OBu^t)-ONSu to give Z-Glu(OBu^t)-Phe-Ala-Ala OMe (IX), crystallized from EtOAc, mp 156–157°, $(\alpha)_D^b$ −31°, yield 72%. IX was converted to Z-Glu(OBu^t)-Phe-Ala-Ala-N_2H_3 (X), crystallized from MeOH, mp 220–221°, $(\alpha)_D^b$ −20°, yield 85%, by treatment with $NH_2.NH_2.H_2O$ (98%).

VII was treated with H_2/Pd black and the free base condensed with the azide from (X) to yield Z-Glu(OBu^t)-Phe-Ala-Ala-Glu(OBu^t)-Glu(OBu^t)-Ala-Ala-Ser(Bu^t)-Phe-OBu^t (XI), recrystallized from DMF, mp 249–250° dec., $(\alpha)_D^{25}$ −19.4° (C = 1, DMSO), yield 76%. The protected decapeptide XI was chromatographically homogeneous and after HCl hydrolysis, its amino acid composition was found to be 0.91 Ser, 3.03 Glu, 4 Ala, 1,95 Phe in an amino acid analyser. Treatment of XI with HBr/TFA yielded I.HBr, recrystallized from DMF-Et_2O, mp 213–214° dec., $(\alpha)_D^{25}$ −59° (C = 1, $CF_3.CO.CF_3.3H_2O$).

Decapeptide I was examined for its ability to hydrolyse chitin and dextran. For this purpose, it was converted into a trisodium salt and dispersed in 0.01 *M* phosphate buffers. Figure 2 shows the rate of release of reducing sugar, measured colorimetrically by the PARK and JOHNSON method[9], on treatment of a suspension of chitin in phosphate buffer with the decapeptide and with hen egg-white lysozyme at pH 5.8 and 7.0. Decapeptide I also hydrolysed dextran at about the same rate as it hydrolysed chitin. The observed activity of decapeptide I is significant but of a low order. Ideal conditions for studying the enzymatic activity of such peptides have, however, still to be established.

The observation of glycosidase activity suggests that the decapeptide has an α-helical conformation. This view is supported by the IR-spectra of the compound. N-H stretching is at 3270 cm^{-1} both in KBr and in dimethyl formamide solution and suggests that only intramolecular

[8] K. HOFMANN, R. SCHMIECHEN, R. D. WELLS, Y. WOLMAN and N. YANIHARA, J. Am. Chem. Soc. *87*, 611 (1965).

[9] J. T. PARK and M. J. JOHNSON, J. biol. Chem. *181*, 149 (1949).

H-bonds are present. The α-helical conformation of the decapeptide I is further evidenced by the difference in ^3H counts given by the peptide after ^3H labelling in presence and the absence of TFA (Table). Treatment of I.HBr in $CF_3.CO.CF_3.3H_2O$ with ^3H_2O was carried out for various time intervals, the residue after complete removal of solvents dissolved in HCOOH and counted using dioxane-based liquid scintillant[10]. In one experiment tritiation of the peptide was done in the presence of TFA in order to permit ready exchange of all the 14 exchangeable protons in the molecule as a result of complete disruption of intramolecular hydrogen bonding. In the helical form, however, 6 of the total exchangeable protons in the peptide can exchange much faster with ^3H than the others involved in hydrogen bonds. The difference in the incorporation of ^3H in the decapeptide with its conformation intact and in presence of TFA is of that order and supports the α-helical conformation of the peptide.

Zusammenfassung. Auf Grund theoretischer Überlegungen sollte das Dekapeptid Glu-Phe-Ala-Ala-Glu-Glu-Ala-Ala-Ser-Phe Glykosidase-Aktivität besitzen. Das synthetische Produkt bestätigt diese Erwartung sowohl gegenüber Chitin als auch Dextran.

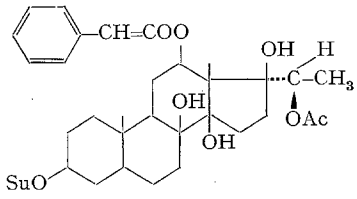
P. K. CHAKRAVARTY, K. B. MATHUR and M. M. DHAR

Central Drug Research Institute, Box 173, Lucknow (India), 28 June 1972.

[10] G. A. BRAY, Analyt. Biochem. *1*, 279 (1960).

Isolation and Structure of Amplexoside A. A New Glycoside from *Asclepias amplexicaulis*[1]

During recent years we have undertaken the isolation and characterization of potential antineoplastic agents from northern Illinois plants as part of an overall program directed toward a chemical investigation of plants in this area. One of our first extracts to show cancer inhibitory activity in the KB assay[2] (ED_{50} 2.3) was from the roots of *Asclepias amplexicaulis*, a milkweed common to many sandy areas of Illinois. This milkweed is, in fact, the first one native to the United States to show this type of activity. We now wish to describe the identification of amplexoside A (I), a major compound (70%) in one of the more active fractions (ED_{50} 0.24) obtained in our isolation work.



Su = Digitoxosyl and asclepobiosyl moieties

Materials and methods. The fatty materials (6.4 g) were first removed from the powdered *Asclepias amplexicaulis* roots (370 g) with hexane. The material was then treated several times with warm alcohol, and the residue from this extract was partitioned between chloroform and water. The chloroform soluble material (9.8 g) was separated in a 10-tube Craig system employing hexane: water: acetone: *t*-butyl alcohol (5:2:4:4). The last 3 bottom layers were combined (3.2 g) and chromatographed on silica gel to yield 20 major fractions upon elution with varying amounts of benzene-acetone. The 2 most active fractions (ED_{50} 0.8 and 2.4) were individually separated on silica TLC plates (41:9 benzene: methanol), and 111 mg of material with ED_{50} 0.24 was collected. Preparative paper chromatography[3,4] (chloroform/formamide) indicated the presence of 1 major compound (70%) and 4 minor ones.

Final purification was achieved by chromatography on an alumina column (neutral, activity III). After removal of the minor components from the column with chloroform, the major compound was recovered with methanol. Two recrystallizations of the eluate residue from chloroform: ether: cyclohexane (1:5:6) produced colorless grains[5] of amplexoside A (I); m.p. 258–260° (sintering at 151–153°); $[\alpha]_{364}^{25}$ 183 ± 3, $[\alpha]_{436}^{25}$ 85 ± 2, $[\alpha]_{546}^{25}$ 43 ± 2 (c, 0.13 in $CHCl_3$). *Anal.* Calcd. for $C_{52}H_{76}O_{18}$: C, 63.12; H, 7.68. Found: C, 62.85; H, 7.03.

The glycoside (3 mg) was hydrolyzed[6] with 0.05 *N* H_2SO_4 in 80% aqueous dioxane into a sugar fraction (1.5 mg) and acylgenin (1.9 mg), which subsequently gave sarcostin (1.3 mg) and cinnamic acid (0.4 mg) upon treatment with 5% methanolic KOH[6].

Results and discussion. The structure of amplexoside A as indicated in (I) is based upon the following evidence. Initial spectroscopic data for amplexoside A suggested the presence of acetyl and cinnamoyl moieties. It had ν_{max} 1732, 1709, 1640, 1580, 1500, and 1238 cm^{-1}; λ_{max} (MeOH) 214 (log ε 3.91), 220 (3.85), 277 nm (3.72) with shoulders at 293 and 303 nm. An NMR-spectrum ($CDCl_3$) indicated among others δ 2.14 (3H, s), 6.24 (1H, d, J = 16), 5.35 (1H, broad t, J = 4), 7.37 (5H, m), and 7.67 (1H, d, J = 16). Prominent mass spectral peaks indicative of acetate and cinnamate functional groups were observed at *m/e* 43 and 131. Further evidence was secured from the mass spectral signals of the acylgenin since there were a faint parent ion at *m/e* 554 and other fragments at *m/e* 536 (M–H_2O), 494 (M–AcOH), 476 (M–H_2O–AcOH), 467 (M–CHOAc·CH_3), 406 (M–cinnamic acid), 346 (M–AcOH–cinnamic acid), 131 (cinnamoyl cation), and 43 (acetyl cation). The peak at *m/e* 467 definitely indicated the acetate moiety was at C-20 of sarcostin, thus placing the cinnamate group at C-12.

Verification of an acetate group was achieved from the formation of a ferric hydroxamate derivative[7] with the

[1] a) Part V of Plant Investigations. Part IV, see D. M. PIATAK and K. A. REIMANN, Tetrahedron Lett., *1972*, 4525; b) This work was supported by grants from the American Cancer Society (IC-26) and its Illinois Division (Seiffert Trust Fund).

[2] KB analyses were performed under the auspices of the Drug Research and Development Banch, National Cancer Institute, according to procedures described in Cancer Chemother. Rept. *25*, 1 (1962). The ED_{50} values represent μg/ml.

[3] L. SAWLEWICZ, EK. WEISS and T. REICHSTEIN, Helv. chim. Acta *50*, 504 (1967).

[4] E. ANGLIKER, F. BARFUSS and J. RENZ, Helv. chim. Acta *41*, 479 (1958).

[5] The purity of amplexoside A was determined in two paper chromatography systems.

[6] L. SAWLEWICZ, EK. WEISS and T. REICHSTEIN, Helv. chim. Acta *50*, 530 (1967).

[7] F. FEIGL, *Spot Tests in Organic Analysis*, 6th edn (Elsevier, New York 1960), p. 250.