with ether and centrifuged. The solid was applied to a silica gel short column and eluted with a chloroform/methanol gradient of 20:1/4:1 and the product zone freeze-dried from dioxane/water to give 155 mg (55%). Further purification was carried out by TLC over silica gel to give **2** as light yellow crystals (mp. >100 °C dec.) from dioxane/water.

Physical data. ^{1}H-NMR (d_6DMSO/TMS = 0) 5.52 and 5.54 (2H, d), 5.88 (1H, s), 6.30 (1H, s), 6.68 (2H, s), 7.8 (4H, m) and 10.78 (1H, s). ^{31}P-NMR (d_6DMSO/85% H_3PO_4 = 0) +4.67 e and +5.91 a. UV (Methanol) ε_{256} = 4.26 TLC (chloroform/methanol 4/1) 0.31, (benzene/isopropanol 2/1) 0.24, comparable to thymidine.

$C_{17}H_{17}N_6O_9P$ (480.3) calculated: C 42.51 H 3.57 N 17.50 P 6.46
found: C 42.33 H 3.85 N 17.22 P 6.27.

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Microbiological transformation of biflavone[1]

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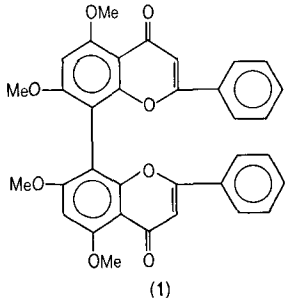
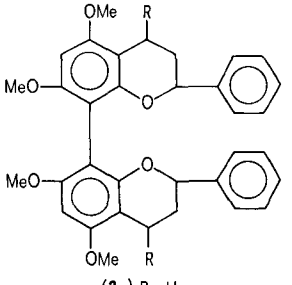
Summary. Incubation of 5,5′-7,7′-tetramethoxy-8,8′-biflavone with *Aspergillus niger* results in the formation of 4,4′-dihydroxy-5,5′-7,7′-tetramethoxy-8,8′-biflavan.

Biflavans with 4,4′ linkage and diol groupings have been synthesised by reductive dimerization of the corresponding flavanones[2]. Biflavans with –4,8– linkages are easily formed by the condensation of the respective flavan-4-ols[3]. So far biflavans with the linkage between the ring A of 2 flavan units are not known. In the present communication, we wish to report the formation of the biflavan (**2a**) from the biflavone (**1**) by the fungus *Aspergillus niger*. The fungus *Aspergillus niger* was cultured in the modified Czapeck-Dox medium[4,5] without substrate for 25 h. 5,5′-7,7′-tetramethoxy-8,8′-biflavone[6] (0.1% in alcohol) was added and incubation continued for an additional period of 103 h at 29 °C. At this time the mycelial mass was acetonised and then extracted along with the culture filtrate, with ethyl acetate. From the ethyl acetate extract compound (**2a**) was isolated in 7% yield as colourless amorphous powder (m.p. 150 °C, ν3460 cm^{-1}), besides other unidentified products by column chromatography on silica gel. The disappearance of the carbonyl group during the fermentation and the emergence of the hydroxyl group, and the characteristic colour reactions, strongly suggest that the product is a biflavan. It was acetylated and the diacetate (**2b**) was used for a detailed study (m.p. 141–143 °C, ν1780 cm^{-1}).



(1)



(**2**) R = H
(**2a**) R = OH
(**2b**) R = $OCOCH_3$

The NMR-spectrum of the biflavan diacetate (**2b**) showed the following signals: δ $CDCl_3$: 6.7 (s,2H) assigned to the protons at the 6,6′ position, 2.8 to 2.5 (m,4H) to the methylene protons, 5.6 to 4.7 (m,4H) to the methine protons, 3.7 (s,6H) to the 2 methoxy groups, 3.8 (s,6H) to the 2 methoxy groups, 7.0 to 6.8 (m,10H) to the protons of ring B and 2.5 to 1.6 (m,4H) to the protons of the 2 acetoxy groups. From the absence of ketonic absorption in the IR-spectrum of the compound (**2a**) and the NMR-spectrum of the compound (**2b**), it is concluded that *Aspergillus niger* metabolized 5,5′-7,7′-tetramethoxy-8,8′-biflavone by the reduction of the carbonyl group and the double bond at C_2 and C_3.

1 Part of this work was done at Allahabad University, Chemical Laboratories.

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Transition metals in calf thymus deoxyribonucleoprotein

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Summary: Fe, Ni, Cu and Zn were found by energy-dispersive X-ray fluorescence in calf thymus deoxyribonucleoprotein. The X-ray analyses indicated the absence of Cr, Mn and Co.

We are reporting the results of an X-ray fluorescence analysis of Cr, Mn, Fe, Co, Ni, Cu, Zn and Ga in native deoxyribonucleic acid (DNA)-chromosomal protein complex, deoxyribonucleoprotein (DNP), extracted from calf thymus by a modification of Messineo's method[2–4].

The importance of transition metals in the function of conjugated biological molecules is well established. The presence of transition metals in deoxyribonucleic acid (DNA) has been reported[5–8]. Wacker and Vallee[5] have reported the emission spectrographic analysis of Cr, Mn,

Fe, Cu and Zn in DNA extracted from bovine liver. The results they obtained for DNA extracted by 2 different methods, the 10% sodium chloride method[9] and the phenol method[10,11], were compared, and substantial differences were observed in the amounts of metal recovered.

Belokobyl' skii et al.[6] and Andronikashvili et al.[7,8] have reported the neutron activation analysis of Cr, Mn, Fe, Cu, and Zn in DNA extracted from various tissues by Georgiev's method[12], and sufficient evidence was presented to show that the Zn was native to the DNA and not the result of contamination[7]. However, the only study of transition metals in DNP is that of Heath[13], who reported values of 77 and 125 ppm for Zn in DNP. The Zn was determined polarographically in ashed samples of DNP extracted from calf thymus by the method of Mirsky and Pollister[14].

The methods used for DNA extraction expose the samples to high salt concentrations to attain separation of proteins from DNA. Exposure of DNP to high salt concentrations during extraction damages the native conjugation[15], and the removal of proteins from the DNP is accompanied by conformational changes in the protein and DNA[16,17]. These procedures may result in some contamination or loss of the native metals. Messineo's method[2-4] for DNP extraction was selected because it seems to achieve a more native soluble preparation consisting of 70% protein and 30% DNA, and the procedure at no time exposes the sample to high salt concentrations or strong chelating agents.

Material and methods. The DNP was extracted from the thymus of 3 calves. Fresh calf thymus was brought to the laboratory on ice and immediately processed with a cell separator to free the cells from the membranes and fibres. The cells were briefly homogenized in 0.01 F $KHCO_3$ and centrifuged at 7000 × g for 20 min. The pellet was washed twice by suspension in 0.01 F glycine and centrifuged at 7000 × g for 20 min. The pellet was extracted by suspension in 0.01 F glycine for 48–72 h. The extract was centrifuged at 25,000 × g for 30 min and filtered through a Whatman 41 filter. The temperature was maintained at 4 °C throughout the extraction procedures. The glycine was removed by gel filtration through a P-10 gel column with 0.01 F NH_4HCO_3 buffer at pH 7.0.

2 internal standards, Cr and Ga, which were not found in either the DNP or thymus cells were added to the DNP solution after gel filtration. For the analysis of thymus cells, the 2 internal standards were added to lyophilized thymus cells and mixed until a homogeneous slurry was obtained. Both types of samples were subsequently lyophilized and pressed into 30–50 mg pellets for metal analysis.

The metals in the sample pellets were analyzed by direct X-ray fluorescence techniques with an energy dispersive X-ray spectrometer modified with cyclindrical graphite monochromators for the incident and fluorescent X-rays[18-20]. The minimum detection limit for the metals in DNP was found to be approximately 0.2 ppm. The metals were quantitated on the basis of the added internal standards by calibration curves and calculations based on a modification of fundamental parameter techniques[19,20]. The reliability of the fundamental parameters calculations was verified by good agreement between the results obtained and those reported by the National Bureau of Standards (USA) for the metals in Bovine Liver No. 1577 and Orchard Leaves No. 1571 reference materials. The buffers used for extraction were analyzed for contaminating materials, and only Cu was found at 6 ppb. The gel filtration buffer was found to contain 21 ppb Cu after elution from the column.

Results and discussion. As shown in table 1, only Fe, Cu, Zn, and a trace of Ni were detected in the 3 DNP samples. The results of the metal analysis of the cells obtained from each thymus used for DNP extraction are shown in table 2. Nickel was below the detection limit in the thymus cells. Cr, Mn, Co and Ga were analyzed but not detected in either the DNP or the thymus cells. A comparison of the results in tables 1 and 2 provides some indication of the metal distribution in the thymus cells.

Table 1. Trace metal analysis of calf thymus DNP

Metal	Amount found (ppm)* Sample No. 1	2	3	Mean ± SD
Fe	19	28	15	21 ± 7
Ni	t**	t	t	t
Cu	19	15	16	17 ± 2
Zn	89	79	79	82 ± 6

*Each value represents the average obtained from calibration curves and fundamental parameters calculations. **Trace.

Table 2. Trace metal analysis of calf thymus cells

Metal	Amount found (ppm)* Sample No. 1	2	3	Mean ± SD
Fe	43	79	40	54 ± 22
Cu	6	6	6	6
Zn	80	92	83	85 ± 6

*Each value represents the average obtained from calibration curves and fundamental parameters calculations.

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The Zn appears to be evently distributed between the DNP and the surrounding cellular matter. However, considering that approximately 40% of the dry weight of the thymus cells consists of DNP, the results indicate that the surrounding cellular matter contains more than 3 times as much Fe as the DNP. Furthermore, the results show that essentially all of the Cu found in the thymus cells is concentrated in the DNP. Since all of the Cu found in the cells was recovered, apparently there was no significant loss during the extraction.

The values we report for Zn in DNP are consistent with those previously reported by Heath[13]. We did not detect Cr and Mn in calf thymus DNP as previously reported in DNA. The presence of these metals may be specific to the type of tissue.

These studies provide the basis for further investigation into the significance of the metals in DNP and DNA. Further studies are in progress which may reveal the function of transition metals in DNP and provide a better understanding of nucleic acid mechanisms.

Syntheses of amino acids from unsaturated aliphatic carboxylic acid by contact glow discharge electrolysis[1]

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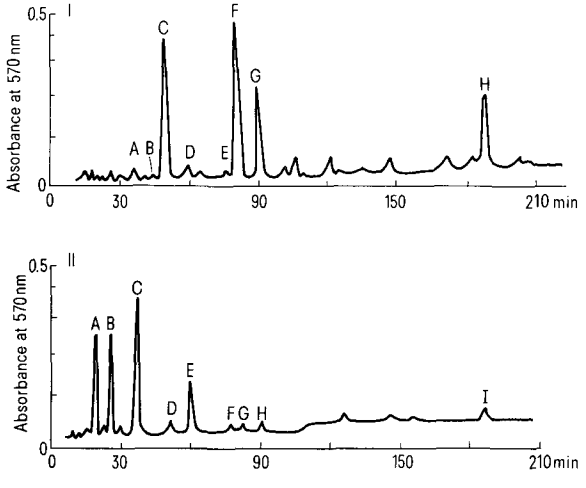
Summary. Hydroxy amino acids are synthesized from unsaturated aliphatic carboxylic acid using aqueous ammonia under conditions of contact glow discharge electrolysis.

Contact glow discharge electrolysis (CGDE) is a chemical change due to the glow discharge in a solution containing ions and an electrode in contact with the solution. Many reactions by CGDE have been studied mainly on inorganic compounds such as water, ammonia and metal ion in an aqueous solution[2,3]. Recently a few studies have been reported on the formation of various amino acids from aliphatic carboxylic acids[4,5] or aliphatic amines[5,6], using aqueous ammoniacal solutions or formic acid solutions, respectively. Urea, glycine and other amino acids were also formed in aqueous ammoniacal solutions by CGDE using a carbon rode as the anode[7].

In the present paper, the synthesis of hydroxy amino acid from unsaturated aliphatic carboxylic acid by CGDE is described. The CGDE was carried out in the reaction tube (a single tube equipped with platinum cathode and anode[5]) containing an ammoniacal solution (about 15 ml) of a substrate (0.005 moles) for 1 h under saturation of ammonia gas while stirring. The applied electric current was 50–60 mA at 400–600 V. The reaction temperature was kept at 10–15 °C by cooling the reaction tube in a methanol-dry ice bath. After the reaction was over, the solution was evaporated to almost dryness under reduced pressure and the residue was diluted appropriately for amino acid analysis (amino acid analyzer: Yanagimoto model LC-5S). The reaction mixture was also treated with 2,4-dinitrofluorobenzene, and the resulting dinitrophenyl (DNP)-amino acids were separated by celite column chromatography[8], followed by identification using a thin-layer chromatoplate. The major amino acid products were identified by comparing the Rf values with the authentic DNP-amino acids.

2 typical charts of the amino acid analyses of the reaction products are shown in the figure. The main amination products of acrylic acid are alanine (Ala, 2.6%) and β-Ala (1.8%). In addition to that, hydroxy amino acids, serine (Ser) and iso-Ser, are also formed in 3.1 and 2.1% yields, respectively. As the control experiments, aqueous ammoniacal solution of acrylic acid was kept at 10 °C for 1 h without CGDE. The amino acid formed in the reaction mixture was only β-Ala (0.2%). The main amino acids synthesized from maleic acid by CGDE are aspartic acid (4.4%), glutamic acid (2.2%), erythro-β-hydroxy aspartic acid (e-OH-Asp, 3.2%) and threo-OH-Asp (2.8%). The results of the amino acid formations from the unsaturated carboxylic acids by CGDE are summarized in the table (reactions No. 1–5), being compared with that of amino acid formation from the corresponding saturated aliphatic carboxlic acids (reactions No. 6–9). In the control experiments in reactions No. 2–5, no amino acids were



Aminations of acrylic acid and maleic acid by CGDE. I. Reaction of acrylic acid. *A* Aspartic acid (Asp); *B* threonine (Thr); *C* serine (Ser); *D* glutamic acid (Glu); *E* glycine (Gly); *F* alanine (Ala); *G* α-aminobutyric acid (α-ABA); *H* β-Ala. Other peaks are of unknown amino acids. Iso-Ser was analyzed by basic column. II. Reaction of maleic acid. *A* erythro-β-Hydroxyaspartic acid (e-OH-Asp); *B* threo-β-hydroxyaspartic acid (t-OH-Asp); *C* Asp; *D* Ser; *E* Glu; *F* Gly; *G* Ala; *H* α-ABA; *I* β-Ala. Other peaks are of unknown amino acids.

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