SPECIALIA

Synthesis and application of the photolabile guanosine 3',5'-phosphoric-*o*-nitrobenzylester

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Summary. The o-nitrobenzylester of guanosine 3',5'-phosphate is prepared and the photolysis studied. This photoactive form of cGMP should be an agent capable of altering the intracellular concentration of cGMP through membrane penetration and subsequent photolysis.

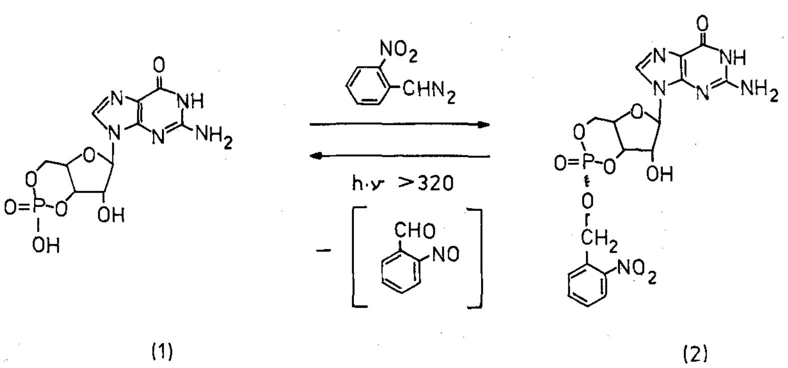
The role which guanosine 3',5'-phosphate (cGMP) plays in the organism is a biological problem which has been intensively studied for years[2]. On the biological level, it is difficult specifically and causally to raise the concentration of cGMP, as can be done in the case of adenosine 3',5'-phosphate (cAMP) by hormone stimulation through noradrenaline. On the chemical level, the analogy to cAMP has excited the intensified search for modified substances which have a special mode of action or are able to give more detailed information about the mechanism of action[3]. One problem with modified substrates is the uncertainty whether they follow the same mechanism as the parent compound. We have attempted in our experiments to do justice to this difficulty by adding a protective group to the biologically active molecule so that it is stable and transportable and can be set free by a specific reaction. One possibility consists of offering a photolabile precursor of cGMP which should be lipophilic and membrane-permeable and generate cGMP upon irradiation by relatively harmless near UV-light (>320 nm)[4]. The o-nitrobenzyl-group[5], which is easily cleaved by UV-light >320 nm, appeared to us to be an appropriate photolabile system. The adenosine 3',5'-cyclophosphate-o-nitrobenzylester fulfilled these requirements in a cell experiment, and the cAMP-dependent protein kinase from bovine brain could be stimulated with the photolysis solution[6]. In this way, a known cGMP concentration should be produceable, depending on the irradiation time.

The synthesis follows the classical method of alkylation of free phosphoric acid with diazoalkanes and was carried out in this case with cGMP and o-nitrophenyldiazomethane, which can be obtained as a crystalline compound in nearly quantitative yields[7] (Scheme).

The o-nitrobenzylester of cGMP is present as a diastereomeric mixture on phosphorus[8]. A separation is in this case extremely difficult (analytic HPLC); this is, however, unnecessary for the photolytical cleavage since it could previously be shown for cAMP-o-nitrobenzylester that both isomers have approximately equal reactivities. It can be shown with the help of the ^{31}PNMR-spectrum that a mixture of 33:67 a:e is present after the synthesis, based on the assumption that the axial compound appears at higher field relative to 85% H_3PO_4[5,8].

The photolysis was carried out in DMSO/H_2O or tris-buffer pH 7.4 (10/1). The reaction was followed with HPLC and the formation of cGMP measured. The speed of the reaction was found to be surprisingly concentration-dependent, a condition which probably must be explained by an intermolecular association of cGMP. The value for 50% cleavage under irradiation at 37 °C from a UV hand lamp (Camag 2 × 20 W) 366 nm at a distance of 6 cm for 10^{-3}–10^{-4} mole **2** amounts to 175 ± 20 min. The hydrolytic stability of the o-nitrobenzylesters was tested in 0.1 N NaOH, 0.1 N HCl and 0.1 N tris-buffer pH 7.4. While the half-life of the triester in base lies in the minute range, the triester is more stable under acidic and neutral conditions, with a half-life of days. The ability of the ester to act as a substrate for phospho-diesterase from beef heart showed a moderate, probably mixed inhibition of 33% for 500 µmole of **1** and **2**.

Preparation. 770 mg (4.7 mmoles) of o-nitrophenyldiazomethane were added to a fine suspension of 194 mg (0.5 mmoles) of cGMP free acid in 10 ml of freshly distilled HMPA and the reaction was stirred under exclusion of light at room temperature. After about 4 days, the reaction was concentrated in vacuum to 1–2 ml, precipitated



(1) (2)

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$_6$DMSO/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$_6$DMSO/85% H$_3$PO$_4$ = 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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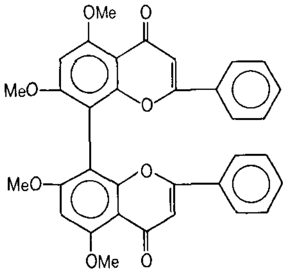
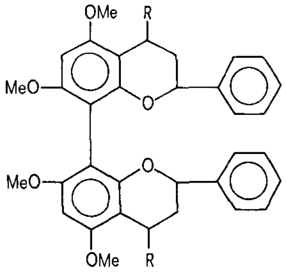
Summary. Incubation of 5,5′-7,7′-tetramethoxy-8,8′-biflavone with *Aspergillus niger* results in the formation of 4,4′-dihydroxy-5,5′-7,7′-tetramethoxy-8,8′-biflavan.

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.

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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,