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.

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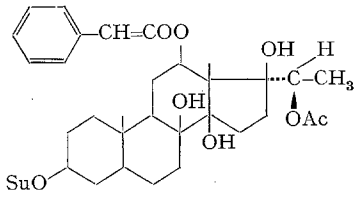
## Isolation and Structure of Amplexoside A. A New Glycoside from *Asclepias amplexicaulis*[1]

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 $\nu_{max}$ 1732, 1709, 1640, 1580, 1500, and 1238 $cm^{-1}$; $\lambda_{max}$ (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).

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acetyl moiety of amplexoside A and comparison of the paper chromatographic characteristics[4] of this derivative to an authentic sample. Final proof for the cinnamate moiety, on the other hand, came about when cinnamic acid was isolated from the hydrolysis reaction of the acylgenin. The isolated cinnamic acid, $\lambda_{max}$ (0.05 *N* KOH) 267 nm; $\lambda_{max}$ (0.1 *N* $H_2SO_4$) 270 nm; $\nu_{max}$ $1698^{-1}$, was confirmed by comparison to a known sample by paper chromatography[8].

The genin portion was established as sarcostin since the isolated material had m.p. 145–150/260–263° (reported[6] m.p. 145–150/257–263°), typical colour reactions with hydrochloric acid[9] and 84% sulfuric acid[10], and the same Rf as a known sample when compared in two TLC systems[11,12] and one paper chromatography system[3].

The sugar portion was placed at C-3 by analogy with other molecules of this type[11]. It was found to consist of asclepobiose and digitoxose by comparison with known specimens in one TLC system[3] and three paper chromatography systems[3,13].

*Résumé.* A partir de la racine d'*Asclepias amplexicaulis* on a isolé un nouveau glycoside de la série prégnane, avec une potentialité d'action anti-cancer. Il possède l'aglycone: 12-cinnamoyl-20-*O*-acétylsarcostine. Les sucres asclépobiose et digitoxose se trouvent á C-3.

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## Activity of Cytidine Triphosphate Synthetase in Normal and Neoplastic Tissues

The pool of free cytidine nucleotides in the animal cell is small compared with those of the other ribonucleotides. Further, it was reported that in tumour cells the amount of these nucleotides is higher than in non-proliferating tissues[1] and that cytidine triphosphate (CTP) decreases to a very low level by the end of postnatal brain development[2]. It may be suggested that CTP, or some step in the CTP producing pathway, is a limiting factor in tissue growth and/or RNA synthesis[1,2].

To test this hypothesis we investigated the activity of CTP synthetase (EC 6.3.4.2, UTP: ammonia ligase (ADP)) in several normal and neoplastic tissues. The presence of this enzyme in animal tissues is proved in soluble extracts from Novikoff hepatoma[3] and liver[3–6].

*Materials and methods.* Liver, kidney, brain, heart, spleen, testis and blood were obtained from adult male rats. Transplantable myeloma MOPC-21 (grown for about 20 days) and Ehrlich ascites cells were obtained from mice, and rhabdomyosarcoma and skeletal muscle from hamsters. Tissues were removed immediately after decapitation of the animals, cut into pieces and rinsed with ice-cold 0.25 *M* sucrose containing 50 m*M* *Tris*-Cl (pH 7.6) and 1 m*M* EDTA. They were homogenized in the same solution with a glass-Teflon homogenizer and the homogenates (25–30%, w/v) were centrifuged in the cold for 80 min at 105,000 *g*. The supernatant fractions obtained were stored in 2 ml aliquots at −20 °C, and were used within 3 weeks after preparation. During this storage, the changes of enzyme activity were insignificant.

The enzyme activity was determined in a standard incubation mixture (see [3–6]) which contained in a final volume of 1.0 ml: 20 μmoles $MgCl_2$, 10 μmoles ATP, 0.4 μmole GTP, 5 μmoles L-glutamine, 10 μmoles phosphoenolpyruvate, 0.22 μmole [4-$^{14}$C] UTP ($8.4 \times 10^6$ counts/min/μmole) and soluble cell fraction containing 2–12 mg protein. The components of the mixture were brought to pH 7.5–7.6 with 1 *M* *Tris*. The incubation was carried out at 37 °C and terminated at different time intervals up to 60 min, after which a slightly modified scheme of Hurlbert and Kammen[3,7] for nucleotide hydrolysis, separation and estimation was followed.

The radioactivity was measured in a Packard Tri-Carb Spectrometer with 2.0 ml aqueous samples and 10 ml of a dioxan-based scintillator fluid, counting efficiency being about 60%. Protein was determined according to Lowry et al.[8], using crystalline bovine serum albumin as a standard.

Activity of CTP synthetase in soluble cell fractions obtained from normal and neoplastic tissues[a]

| Tissue | CTP synthetase (nmoles/h/mg protein) |
|---|---|
| Blood | 0 |
| Sceletal muscle | 0.4 |
| Liver | 0.7 |
| Heart | 1.2 |
| Kidney | 1.5 |
| Brain | 1.6 |
| Spleen | 1.8 |
| Rhabdomyosarcoma | 4.0 |
| Myeloma | 5.1 |
| Testis | 12.2 |
| Ehrlich ascites tumour cells[b] | 16.0 |

[a] For the origin of the tissues and isolation of the soluble cell fractions see 'Materials and methods'. The Figures are mean values from 3 to 10 independent samples. [b] The cells were collected 9–12 days after implantation.

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