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CELL GROWTH INHIBITORY GLYCOSIDES FROM *ASCLEPIAS AMPLEXICAULIS*

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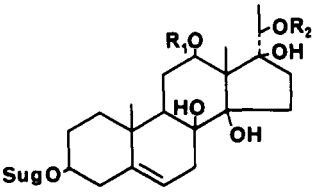
Our prior study (1) of the roots from *Asclepias amplexicaulis* Sm (Asclepiadaceae) resulted in the isolation and identification of amplexoside A (**1**), which had shown cell growth inhibitory activity against the NCI KB cell line from a human nasopharnyx carcinoma (2). In this report, we wish to present tentative structures for two additional glycosides from this plant, namely, amplexoside B (**2**) and amplexoside C (**3**), which were found to have KB cell line ED_{50} values (2) of 3.9 and 0.14, respectively. These results together with our previous study (1) and with results reported by the Mitsuhashi group (3) further establish the pregnane glycosides as interesting steroidal cytotoxic agents.

From the essentially identical ir and uv spectra of amplexosides B and C and their similarity to those of amplexoside A, the two glycosides were assumed to be pregnane glycosides containing at least a cinnamate ester function. The noncrystalline aglycone isolated after acid hydrolysis of both glycosides was established as the same for both by tlc. The structure **4** was assigned from identical nmr spectra which had, among others, signals at δ 1.12(s), 1.23(d), 1.55(s), 4.68(q), 4.82(q), 5.32(m), 6.23(d), 6.76(m), and 7.54(d) in accordance with values reported by Hayashi and Mitsuhashi (4) for this aglycone. The tiglate and cinnamate moieties were confirmed by field ionization ms wherein signals appeared at *m/z* 55, 83, 103, and 131 corresponding to these units. After base hydrolysis, the cinnamic acid was reconfirmed from its spectral data, and sarcostin (**5**) was authenticated as the steroidal portion of both glycosides.



1 $R_1=C_6H_5CH=CHCO-$; $R_2=Ac$; Sug=asclepobiosyl, digitoxosyl
2 $R_1=C_6H_5CH=CHCO-$; $R_2=MeCH=CMeCO-$; Sug=cymarosyl, digitoxosyl
3 $R_1=C_6H_5CH=CHCO-$; $R_2=MeCH=CMeCO-$; Sug=rhamnosyl, cymarosyl, digitoxosyl
4 $R_1=C_6H_5CH=CHCO-$; $R_2=MeCH=CMeCO-$; Sug=H
5 $R_1=R_2=Sug=H$

The sugar portion of both glycosides was assumed to be on C-3 by analogy to other molecules of this type (5,6). Tlc of the sugars from amplexoside B (**2**) on silica gel and cellulose sheets with three separate solvent systems (7) indicated three sugars, two of which corresponded to authentic cymarose and digitoxose while the third was not identified. A fims had two distinct series of peaks at *m/z* 162, 144, and 126 and *m/z* 148, 130, 112, and 94 which confirmed the presence of only cymarose and digitoxose. Therefore, the third sugar seen by tlc is likely a disaccharide of these two. While very weak peaks supporting a trisaccharide glycoside were observed above *m/z* 576, it would need to contain two units of one of the two identified

[1]Deceased.

monosaccharides. In addition, prominent peaks were observed at *m/z* 576, 446, 428, and 410. The latter three can be attributed to the aglycone portion from which cinnamic acid, H_2O, and another H_2O molecule are consecutively expelled, while the former peak could represent either loss of H_2O from the aglycone only or loss of cinnamic acid from a combination of the aglycone and the first sugar attached. Should the latter presumption be correct, then the sugar directly attached to the aglycone would be digitoxose.

For amplexoside C (**3**), the sugars corresponded to cymarose, digitoxose, and rhamnose in three different tlc systems on silica gel and cellulose sheets (14). A fims had three distinct series of diagnostic peaks at *m/z* 162, 144, and 126; at *m/z* 148, 130, 112, and 94; and at *m/z* 128 and 110 which helped confirm the identity of these monosaccharides. A peak at *m/z* 274 corresponded to a combination of cymarose and digitoxose minus a H_2O molecule. The higher mass portion of the spectrum, again, had no prominent peaks except at *m/z* 576, so the saccharide sequence could not be firmly established. However, by analogy to the presumption made for amplexoside B, digitoxose could be attached at C-3 of the aglycone, which would place rhamnose at the terminus.

EXPERIMENTAL

ISOLATION.—Roots of *A. amplexicaulis* were collected in Ogle County, Illinois, by D.M. Piatak and P.D. Sørensen. A voucher specimen is available in the Northern Illinois University Herbarium.

Fresh $CHCl_3$-soluble material (15.4 g) obtained as before (1) was treated with $Pb(OH)_2$ in MeOH (8) to afford 6.64 g of material which was chromatographed on a medium pressure silica gel column (EM silica gel 60 less than 230 mesh) with $CHCl_3$. Elution was continued (10 ml fractions) with $CHCl_3$ and $CHCl_3$ containing increasing amounts of MeOH (750 fractions total). Fractions were combined on the basis of tlc data. Amplexoside B (12.5 mg; mp 166-168°) was crystallized from fractions 280-380 and amplexoside C (5.0 mg; mp 173-175°) from fractions 600-750 by MeOH-$CHCl_3$. Both samples had ir ν max (KBr) 3400, 1705, 1640, 1580, 1500 cm^{-1} and uv λ max (MeOH) sh 210, 217, 221, 280 nm.

HYDROLYSIS OF GLYCOSIDES.—Acid hydrolysis of both glycosides with 0.1 N HCl in MeOH and separation into aglycone and sugar fractions was accomplished as described (9) for other glycosides. Both noncrystalline aglycones **4** had identical Rf values on silica gel HF_{254} (E. Merck 0.25 mm sheets) and cellulose F_{254} (E. Merck 0.1 mm sheets) with $CHCl_3$-MeOH-H_2O (80:18:2) and *n*-BuOH-HOAc-H_2O (60:20:20), respectively. ^{1}H-nmr and ms data for **4** and identification of the sugars are described in the text.

The aglycone **4** was hydrolyzed by base (10) to give sarcostin (**5**) which was identical by two tlc systems (5,11) on silica gel and by spectral comparison (ir, ^{1}H nmr) to an authentic sample and cinnamic acid which was identified from its uv and ^{1}H-nmr spectra.

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