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SPECIALIA

Adina Alkaloids: The Structure of 5-Oxostrictosidine

Further fractionation of the neutral glycosides from *Adina rubescens* heartwood has afforded in low yield (4 mg/0.4 kg) a new alkaloid which has been assigned the structure of 5-oxostrictosidine (**1**) on the basis of chemical and spectroscopic evidence outlined below.

It was isolated and characterized as the amorphous acetate derivative A, $C_{37}H_{42}N_2O_{15}[\alpha]_D^{52}$-20° ($CHCl_3$), and since the corresponding propionate differed by 70 m.u. contained 5 acylable groups. Catalytic hydrogenation of the penta-acetate gave the dihydro compound $[\alpha]_D^{25}$ −23° ($CHCl_3$). Indole and β-alkoxyacrylate functions suggested by UV [λ_{max} 228, 282, 290 nm; λ_{min} 259 nm] and IR [$!_{max}$ 3400, 1705, 1630 cm^{-1}] spectra were confirmed by signals in the NMR spectrum at τ − 0.85 (indolic NH), 2.2–2.9 (4 aromatic H), 2.59 (O=C−C=CH−O) and a spike at τ 6.38 extended the latter to a methyl ester. 3 olefinic protons between τ 4.1 and 4.6 were consistent with a vinyl group, which was substantiated when the presence of a new ethyl group in the dihydro derivative was established by a three-proton NMR triplet at τ 8.92. 4 acetate singlets from τ 7.81 to 8.07 and a typical absorption pattern between τ 4.40 and 6.15 (H'1–6') indicated a hexapyranoside tetra-acetate moiety.

Detailed examination of the mass spectra together with mass measurement revealed many features of the structure. In addition to a series of ions at *m/e* 423 (M^+-$C_{14}H_{19}O_9$), 407(M^+-$C_{14}H_{19}O_8$), 331($C_{14}H_{19}O_9$), 169 and 109 due to the acetylated hexoside, an ion at *m/e* 165($C_9H_9O_3$) which moved to *m/e* 167 after hydrogenation could readily be attributed to the pyrylium ion **7** by analogy with known alkaloids[1]. The observed ready loss of CH_3CO from the molecular ion (M^+754) to give an intense peak at *m/e* 711 together with a metastable at 670.4 was typical of an N(b)-acetamide, which also accounted for the 5th acetyl group, even though its chemical shift (τ 7.30) was at apparently low field. 2 other strong ions at *m/e* 227 ($C_{13}H_{11}N_2O_2$) and 185 ($C_{11}H_9N_2O$, base peak) corresponded to typical β-carboline fragments except that they contained an extra oxygen atom, which the evidence above restricted to ring C as in **8**.

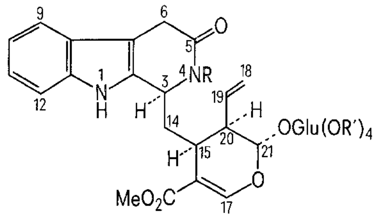
Further evidence about this oxygen function was obtained when Zemplen *O*-deacetylation with NaOMe in MeOH, followed by brief reacetylation with Ac_2O/py did not give the starting material but 3 new amorphous products B, C and D separable by TLC. The formula ($C_{35}H_{40}N_2O_{14}$) of B, M^+ 712, corresponded to loss of an

100 MHz NMR-spectra

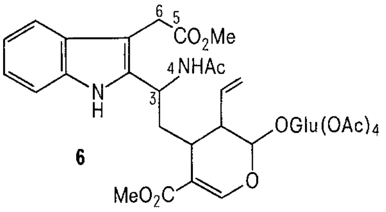
Structure	**2** [$(CD_3)CO$]		**4** [$CDCl_3$]		**5** [$CDCl_3$]
Proton	τ	J (Hz)	τ	J (Hz)	τ
1-H	−0.85s		1.06		1.41
3-H	3.94m	7.5, 4.5, 1	4.42m	7.5, 4, 1	?
6-H_2	5.90d	1	6.09d	1	?
9-12-H	2.2–2.9m		2.4–3.0m		2.4–3.0m
14-H_a	7.54m	4.5, ?, ?	?		?
14-H_b	7.42m	7.5, ?, ?	?		?
15-H	7.09m	2, ?, ?	7.14m	2, ?, ?	ca. 7.1
17-H	2.59d	2	2.81d	2	2.47s
18-H_2	4.3–4.6m		8.92t	6.5(CH_3)	ca. 4.8
19-H	4.1–4.3m		8.25m	6.5, ?(CH_2)	4.25
20-H	7.58m	3.5, ?, ?	?		ca. 7.5
21-H	4.68d	3.5	4.56d	3.5	4.5–5.4
22-OMe	6.38s		6.67s		6.47s
NAc	7.30s		7.47s		—
17-OMe	—		—		6.53s
1'-4'-H	4.4–5.0m		4.5–5.2m		4.5–5.4
5'-H	6.15m		6.2m		ca. 6.2
6'-H_2	5.6–5.9m		5.7–5.8m		5.7–6.0m
$(OAc)_4$	7.81–8.07s		7.90–8.20s		7.74–7.98s

acetate function, presumably from N(b) since strong M-43 and *m/e* 227 ions were no longer present in its mass spectrum. Mass and NMR spectra showed that a similar loss of a N-acetyl group had occurred in compound C, M+ 744 ($C_{36}H_{44}N_2O_{15}$) together with addition of a molecule of MeOH, which the lack of UV-absorption at 238 nm indicated was to the *β*-alkoxyacrylate chromophore. Although the molecular formula ($C_{38}H_{46}N_2O_{16}$) of the 3rd compound D, M+ 786, was consistent with a simple addition of MeOH to the starting material, its UV-spectrum still retained the acrylate absorption and its mass spectrum differed markedly from the others. No *β*-carboline fragments were apparent, the predominant losses being of 58($NHCOCH_3$) and 59(CO_2CH_3) m.u. from the molecular ion, together with lesser ones of 32(CH_3OH) and 43(CH_3CO). This behaviour was compatible with structure **6** for substance D, where the ions **9** and **10** could be generated via the favoured 3–4 and 5–6 cleavages respectively. Corresponding structures for B and C would then be the lactams **3** and **5**.

A parallel Zemplen deacetylation of compound A followed by propionylation showed that in all 3 products only the sugar was re-acylated, and that only C had retained the original acetamide linkage. Under the mild reaction conditions (a catalytic amount of NaOMe in MeOH at room temperature) a normal amide would remain intact. The above behaviour of compound A could be rationalized by the imide structure **2**, in which N-4 is attached to 2 carbonyl groups, each of which is more reactive. Thus nucleophilic attack by MeO(−) on the N-acetyl would result in deacetylation to the stable lactam, whereas an alternative attack on the C-5 carbonyl would open the ring to form an acetamide. Non-regeneration of A from B on re-acetylation is attributable to the brevity of the reaction – when treatment is prolonged (16–24 h) as in the original isolation procedure, the lactam is eventually acylated.



	R	R'	
1	H	H	
2	Ac	Ac	
3	H	Ac	
4	Ac	Ac	18,19-dihydro



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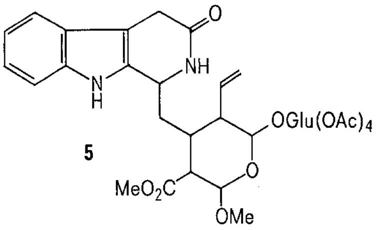

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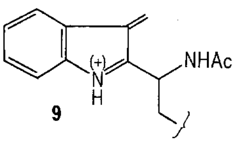

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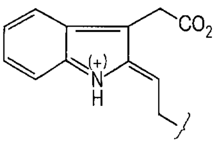

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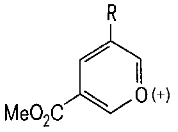

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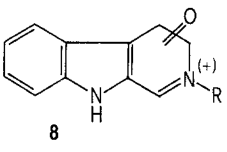

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The nature of the sugar was established when *β*-glucosidase cleaved the deacetylated compounds to the corresponding aglucones, all of which on addition of alkali showed the expected UV-shift to 275 nm due to ionization of a *β*-hydroxyacrylate chromophore.

Confirmation of structure **2** for A was afforded by a detailed analysis of the NMR-spectrum (see Table) with the aid of spin decoupling which enabled virtually all the protons to be located and also provided some stereochemical information. Thus H-20 was coupled to H-21, 19 and 15; H-15 had an allylic interaction with H-17, and was also shown to be coupled to the methylene protons on C-14, whicl in turn were coupled to H-3; more importantly, assignment of the C-6 methylene group was facilitated by a small long-range interaction with H-3. Moreover, the presence of a diacylamine function offered a satisfactory explanation for the low-field acetate signal at τ 7.30.

Since some of the signals were obscured not all of the coupling constants could be obtained, but the 20–21 and 3–14 couplings were comparable to those of compounds related to strictosidine[2] and suggested a similar stereochemistry. More substantial support for this came from the CD-spectrum of the penta-acetate ($[\theta]_{295}$ + 1.4×10^4, $[\theta]_{276}$ + 3.1×10^4 deg. cm²/decimole) where the positive Cotton effect established the absolute configuration at C-3 as α(S) by analogy with tetrahydro-*β*-carboline acetamides of known chirality at C-3[3]. Biogenetic considerations would dictate that in all likelihood the remaining stereochemistry is the same as in secologanin, and hence the new alkaloid can be formulated as 5-oxostrictosidine (**1**).

Zusammenfassung. Für ein neues glukosidisches Indolalkaloid aus *Adina rubescens* wird anhand instrumentalanalytischer Untersuchungen die Struktur von 5-Oxostrictosidin vorgeschlagen.

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[1] R. T. Brown and L. R. Row, Chem. Commun. *1967*, 453.

[2] K. T. D. De Silva, G. N. Smith and K. E. H. Warren, Chem. Commun. *1971*, 905.

[3] R. T. Brown, unpublished results cited in M. Koch, M. Plat and N. Preaux, Bull. Soc. chim., Fr. *1973*, 2868.

Synthesis of Litorin

A nonapeptide of the formula H-Pyr-Gln-Trp-Ala-Val-Gly-His-Phe-Met-NH_2[1], corresponding to the proposed sequence of litorin[2], was synthesized by conventional methods. Relevant information pertaining to its synthesis is summarized in the Figure and in the Table.

The free nonapeptide, after the removal of the dinitrophenyl group with a large excess of 2-mercaptoethanol[3] in solution of HMPT-DMF-H_2O (4:1:1) maintained at pH 8 with Na_2CO_3, was finally secured as hydrochloride by treatment with HCl-AcOH and desalting through a column of amberlite XAD-2 (eluent:H_2O and then MeOH-H_2O 50%). It was found to be homogeneous and showed the same electrophoretic and chromatographic

Data on litorin and the intermediates obtained during the synthesis*

Number	Compound	Formula	Method[a]	Reaction[b] solvent	Yield (%)	Crystallization solvent[c]	Melting[d] point	Optical[e] rotation	$E_{1.2}$[f] (Glu)	$E_{5.8}$[g] (Glu)
I	Boc-Phe-Met-NH_2	$C_{19}H_{29}N_3O_4S$	M.A.	THF	70	MeOH-EtOAc	193–195°	—	—	—
II	H-Phe-Met-NH_2·HCl	$C_{14}H_{21}N_3O_2S \cdot HCl$	HCl	AcOH	98	MeOH-Et_2O	210–212°	+14°	0.85	—
III	Boc-His(DNP)-Phe-Met-NH_2	$C_{31}H_{38}N_8O_9S$	DCCI + HOSu	THF-DMF	65	MeOH-Et_2O	135–140°d	− 8°	—	—
IV	H-His(DNP)-Phe-Met-NH_2·HCl	$C_{26}H_{30}N_8O_7S \cdot HCl$	HCl	AcOH	90	MeOH-Et_2O	155–160°d	+30°	0.98	—
V	Boc-Gln-Trp-Ala-Val-Gly-OH	$C_{31}H_{45}N_7O_{10}$	NaOH	MeOH-H_2O	84	MeOH-Et_2O	156–157°	−47°	—	0.32
VI	H-Gln-Trp-Ala-Val-Gly-OH·TFA	$C_{26}H_{37}N_7O_8 \cdot C_2F_3HO_2$	TFA	—	90	MeOH-Et_2O	170–172°	+29°	0.51	—
VII	Z-Pyr-Gln-Trp-Ala-Val-Gly-OH	$C_{39}H_{48}N_8O_{12}$	ONP	DMF	80	AcOH-Et_2O	230–232°d	−52°	—	0.24
VIII	H-Pyr-Gln-Trp-Ala-Val-Gly-OH	$C_{31}H_{42}N_8O_{10}$	H_2	DMF	95	DMF-Et_2O	239–240°d	−43°	—	0.25
IX	H-Pyr-Gln-Trp-Ala-Val-Gly-His(DNP)-Phe-Met-NH_2	$C_{57}H_{70}N_{16}O_{16}S$	DCCI + HOSu	DMF-HMPT	66	DMF-MeOH	180–182°d	−25°	—	—
X	H-Pyr-Gln-Trp-Ala-Val-Gly-His-Phe-Met-NH_2·HCl (litorin hydrochloride)	$C_{51}H_{69}N_{14}O_{11}S \cdot HCl$	M.E. pH 8	HMPT-DMF-H_2O(4:1:1)	60	MeOH-EtOAc	237°d	−22°	0.40	—

Amino acid ratios in acid hydrolysate[h] of compound X (litorin hydrochloride): $Glu_{1.94}$, $Gly_{1.00}$, $Ala_{1.00}$, $Val_{0.95}$, $Met_{1.00}$, $Phe_{0.95}$, $His_{0.99}$. *All compounds (except II, IV and VI, which were not analyzed) gave correct combustion values for C, H and N. [a]M.A., mixed anhydride with N-methylmorfoline and ethyl chloroformate (activation time: 2 min at −15°); DCCI + HOSu, activated ester prepared in situ from N,N′-dicyclohexylcarbodi-imide and N-hydroxysuccinimide (2 h at 0° and 2 h at 24°); HCl, dry HCl (~1,3 *N*); NaOH, 1 *N* sodium hydroxide; TFA, trifluoroacetic acid at 0°; ONP, *p*-nitrophenyl ester; H_2, hydrogenation in the presence of 10% palladium-charcoal; M.E., 2-mercaptoethanol; pH 8, maintained at pH 8 with solid Na_2CO_3. [b]THF, tetrahydrofuran; AcOH, glacial acetic acid; DMF, N,N′-dimethylformami de; MeOH, methanol; H_2O, water; HMPT, hexamethyl phosphoric triamide. [c]EtOAc, ethyl acetate; Et_2O, diethyl ether. [d]d, with decomposition. [e]Optical rotations were measured at 24°, c = 1. The solvents used are MeOH for II, III, IV, V and VI; AcOH for VII, VIII and IX; 95% AcOH for X. [f]Electrophoretic mobility in $HCOOH/CH_3COOH/H_2O$ (615:500:3885) with glutamic acid as standard. [g]Electrophoretic mobility in CH_3COOH/pyridine/H_2O (50:450:4500) with glutamic acid as standard. [h]Trp is decomposed during acid hydrolysis (105° for 16 h).

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[1] The amino acids used, with the exception of glycine, have the L-configuration. Symbols and abbrevations are in accordance with the recomandations of the IUPAC-IUB Commission on Biochemical Nomenclature, J. Biol. Chem. *247*, 977 (1971). Pyr = pyroglutamic acid.

[2] A. Anastasi, V. Erspamer and R. Endean, Experientia *31*, 510 (1975).

[3] F. Chillemi and R. B. Merrifield, Biochemistry *8*, 4344 (1969).