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THE TOXIC EXTRACTIVES FROM *WEDELIA ASPERRIMA*—I

THE STRUCTURE AND SYNTHESIS OF THE UNUSUAL GLYCOSIDIC PORTION OF WEDELOSIDE

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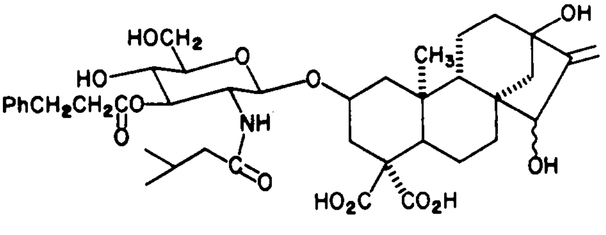
Abstract—The major toxic constituent of the plant, *Wedelia asperrima*, is the diterpene aminoglycoside, wedeloside. MS and NMR analysis of the unusually substituted aminoglycosidic portion of wedeloside and comparison with synthetic analogues have permitted the assignment of its structure as a 2 - deoxy - 2 - (3 - methyl - 1 - oxobutyl)amino - 3 - 0 - (1 - oxo - 3 - phenylpropyl) - D - glucopyranoside, β-linked to the diterpene aglycone.

Wedelia asperrima Benth., commonly known as the yellow daisy, is responsible for serious sheep losses in north western Queensland.[1] We have recently reported[2] on the isolation, toxicity and potential anti-tumor activity of the major toxic constituent from *W. asperrima*, which we have called wedeloside **1**. In this paper we describe the definitive structural elucidation of the glycosidic portion of this compound. The determination of the structure of the diterpene aglycone will be reported later.

Wedeloside **1** is unusual in that, as far as we are aware, it is the first reported example of an acylaminodeoxyhexose glycosidically linked to a diterpene. Another uncommon feature of the sugar moeity is the presence of the 3-methyl-1-oxobutyl glycosidic functionality, which has been reported[3–6] in atractyloside **2** and carboxyatractyloside **3** where it is present as an ester rather than an amide. Further, we believe that the 3-phenylpropionyl substituent in wedeloside is a novel structural feature of diterpene glycosides and assume that it arises biogenetically from reduction of a cinnamic acid precursor.

Because of the small amounts of wedeloside initially available and the presence in the extract of closely related compounds as minor impurities, most of the early structural elucidation studies were carried out using mass spectrometric methods. This necessitated the preparation of a number of synthetic analogues and comparison of the mass spectrometric fragmentation patterns of synthetic and natural compounds to allow structural assignments to be made. When larger quantities of wedeloside became available, from the improved extraction procedure described herein, comparisons of ^{13}C and ^{1}H NMR spectra of degradation products and synthetic analogues were used to confirm the proposed structure.



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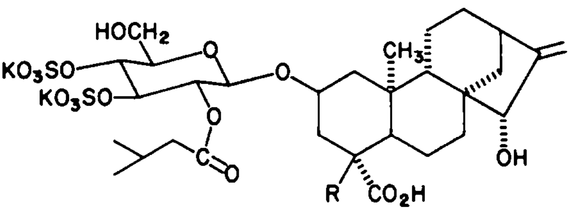


2: R = H
3: R = COOH

RESULTS AND DISCUSSION

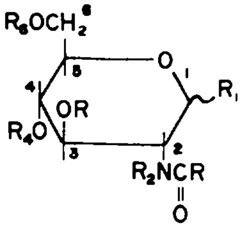
Wedeloside **1** is an amorphous powder, which, because of its involatility and thermal instability, was not amenable to mass spectrometric analysis by direct probe insertion without prior derivatisation.

On permethylation,[7] 1 was converted into 4 whose mass spectrum is shown in Fig. 1a. High resolution accurate mass measurement of the molecular ion ($M^{+\cdot}$) of 4 at *m/z* 737 gave its composition as $C_{39}H_{63}NO_{12}$. The trideuteromethyl analog 5 prepared in a similar manner using CD_3I showed a shift in the $M^{+\cdot}$ to *m/z* 761 (Fig. 1b) indicating an uptake of eight Me groups by wedeloside on permethylation. By a combination of accurate mass measurements on selected ions in the mass spectrum of 4 (e.g. *m/z* 521, 419, 302, 270, 184, 87) and the relative deuterium isotopic shifts of these ions as determined from the spectrum of 5, it was possible to construct a partial structure for 4 as shown (Fig. 2).

Acidic methanolysis of 4 yielded two compounds. The first, compound 7, showed an $M^{+\cdot}$ at *m/z* 436 with the composition $C_{24}H_{36}O_7$ and could be assigned as the aglycone, i.e. the right hand potion of Fig. 2, while the other compound 6 had an $M^{+\cdot}$ at *m/z* 333 which corresponded to the nitrogen containing fragment of 4 (Fig. 2) with an added OMe substituent. The remainder of the discussion will focus on the determination of the structure of the latter portion of the molecule.

The composition of the nitrogen containing moiety showed that it contained only two rings and/or double bonds and that it most likely was a monosaccharide, while the presence of the N atom and an IR absorption max at 1643 cm^{-1} suggested that it could be an acylamino sugar. This was supported by the presence of the nitrogen containing ions at *m/z* 87 and 100 in the mass spectrum of 4 (Fig. 1a) which are prominent in the spectra of permethylated 2 - acetylamino - 2 - deoxyhexoses.[8] Heyns and Müller[8] have reported a detailed study of the mass spectra of the permethylated derivatives of 2-, 3- and 6-acetylaminodeoxyhexoses from which it was possible to correlate certain of the structurally significant ions in the mass spectrum of methyl 2 - acetamido - 2 - deoxy - N - methyl - 3,4,6 - tri - O - methyl - *β* - D - glucopyranoside 8 with ions (e.g. *m/z* 218, 186, 172, 100, 87) in the mass spectrum of 6 (Table 1). Other ions present in the mass spectrum of 6, including $M^{+\cdot}$, were a constant 42 mass units above assigned ions at *m/z* 260, 228, 217, 142 and 129 in the spectrum of 8 (Table 1). This difference, which was mass measured as C_3H_6, was associated with presence of the alkyl substituent R on the 2-amino substituent (R=Me in 8) and strongly suggested that in compound 6 the R group was C_4H_9.

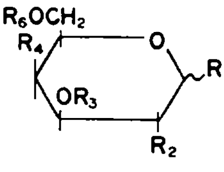
The correlation of the fragmentation patterns of 6 and



6: R_1 = OMe, $R_2 = R_3 = R_4 = R_6$ = Me, R = $-C_4H_9$
8: R_1 = OMe, $R_2 = R_3 = R_4 = R_6$ = Me, R = $-CH_3$
9: R_1 = OH, $R_2 = R_3 = R_4 = R_6$ = H, R = $-\overset{8}{C}H_2\overset{9}{C}H(\overset{10}{C}H_3)(\overset{11}{C}H_3)$

10: R_1 = OH, $R_2 = R_3 = R_4 = R_6$ = H, R = $-\overset{8}{C}H(\overset{11}{C}H_3)\overset{9}{C}H_2\overset{10}{C}H_3$

11: R_1 = OH, $R_2 = R_3 = R_4 = R_6$ = H, R = $-\overset{8}{C}H_2\overset{9}{C}H_2\overset{10}{C}H_2\overset{11}{C}H_3$
12: R_1 = βOMe, $R_2 = R_3 = R_4 = R_6$ = Me, R = $-CH_2CH(CH_3)_2$
13: R_1 = βOMe, $R_2 = R_3 = R_4 = R_6$ = Me, R = $-CH(CH_3)CH_2CH_3$
14: R_1 = βOMe, $R_2 = R_3 = R_4 = R_6$ = Me, R = $-(CH_2)_3CH_3$
15: R_1 = OTMS, R_2 = H, $R_3 = R_4 = R_6$ = TMS, R = $CH_2CH(CH_3)_2$
16: R_1 = OTMS, R_2 = H, $R_3 = R_4 = R_6$ = TMS, R = $-CH(CH_3)CH_2CH_3$
17: R_1 = OTMS, R_2 = H, $R_3 = R_4 = R_6$ = TMS, R = $-(CH_2)_3CH_3$
18: R_1 = OTMS, R_2 = H, $R_3 = R_4 = R_6$ = TMS, R = $-C_4H_9$
19: R_1 = αOMe, R_2 = H, $R_3 = R_4 = R_6$ = TMS, R = $-C_4H_9$
20: R_1 = αOMe, $R_2 = R_3 = R_4 = R_6$ = H, R = $-CH_2CH(CH_3)_2$
21: R_1 = αOMe, R_2 = H, $R_3 = R_4 = R_6$ = TMS, R = $-CH_2CH(CH_3)_2$



22: $R_1 = R_2$ = OMe, $R_3 = R_6$ = Me, R_4 = $N(CH_3)COCH_2CH(CH_3)_2$
23: $R_1 = R_4$ = OTMS, R_2 = $N(H)COCH_2CH(CH_3)_2$, $R_3 = R_6$ = TMS

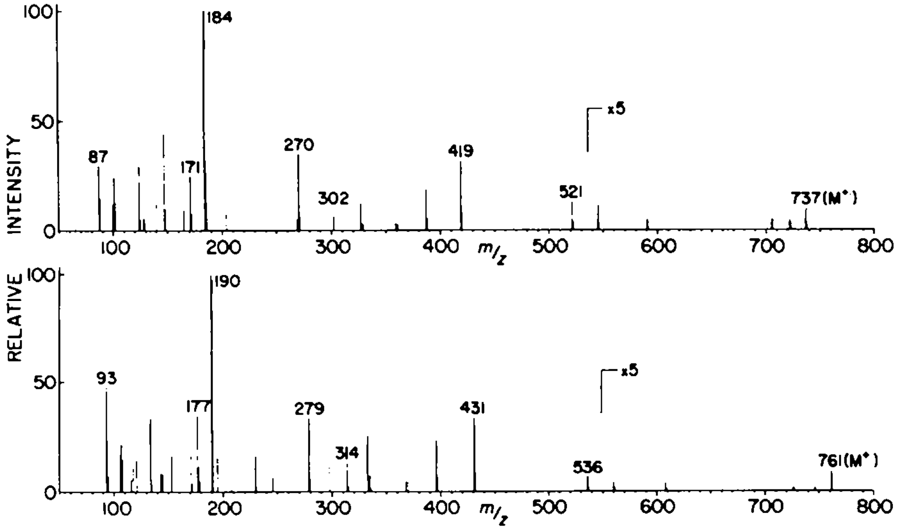


Fig. 1. Mass spectra of the (a) permethylated derivative 4 and (b) pertrideuteromethylated derivative 5 of wedeloside.

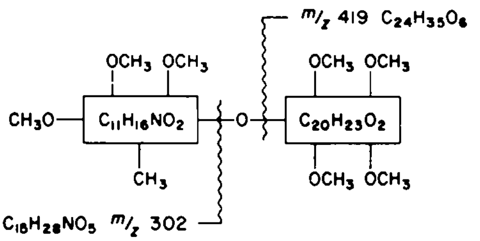


Fig. 2. Partial structure of 4 based on permethylation studies and high resolution mass measurements.

8, coupled with their dissimilarity to the mass spectra of the permethyl derivatives of 3- and 6-deoxyacetamidohexoses,[8] ruled out these latter two positions for the acylamino substituent in 6. On available evidence, it was not possible, however, to eliminate the 4-position as mass spectra of suitable model compounds had not been reported. We therefore synthesised methyl 4 - deoxy - N - methyl - 2,3,6 - tri - O - methyl - 4 - (3 - methyl - 1 - oxobutyl) amino - α - D - galactopyranoside 22 and observed that its mass spectrum differed substantially from that of 6. In particular, high intensity ions were present in the mass spectrum of 22 at *m/z* 158 (60%), 196 (100%) and 241 (46%) which were weak or absent in the spectrum of 6.

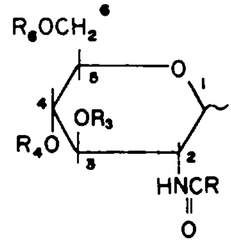
On the other hand, the mass spectrum of authentic methyl 2 - deoxy - N - methyl - 3,4,6 - tri - O - methyl - 2 - (3 - methyl - 1 - oxobutyl) amino - β - D glucopyranoside 12, prepared by permethylation of the synthetic parent compound 9, was virtually indentical with that of 6 (Table 1 for partial spectra). This confirmed that 6, the glycosidic portion of 4, was the permethylated derivative of a 2 - deoxy - 2 - valeramido - hexopyranose which was necessarily linked via C1 to the aglycone. The two major structural features of 6 which remained to be elucidated were (1) the nature of the hexose sugar and (2) the structure of the C_4H_9CO acyl moiety on the 2-amino substituent.

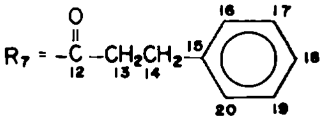
Table 1. Partial mass spectra, *m/z* (% RI), and ion assignments for the permethylated derivatives 6, 8 and 12

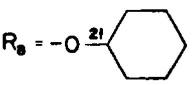
8[8]	6	12	Ion[8]
291 (0.1)	333 (0.1)	333 (0.1)	$M^{+\cdot}$
260 (1)	302 (0.4)	302 (0.3)	$M^{+\cdot} - R_1$
228 (2)	270 (0.3)	270 (0.5)	$M^{+\cdot} - R_1 - MeOH$
218 (4)	218 (1.5)	218 (1.5)	$M^{+\cdot} - R_2NHCOR$
217 (3.5)	259 (0.3)	259 (0.5)	$M^{+\cdot} - R_6OCH_2CHO$
186 (4)	186 (4)	186 (4)	$M^{+\cdot} - MeOH - R_2NHCOR$
172 (2)	172 (2.5)	172 (3)	$M^{+\cdot} - R_1 - R_6OCH_2 - RCO$
142 (48)	184 (33)	184 (33)	$R_4OCH{=}CH\text{-}\overset{+}{C}H\text{-}N(R_2)COR$
129 (40)	171 (5)	171 (8)	$R_3\dot{O}CH\text{-}\overset{+}{C}HN(R_2)COR$
100 (33)	100 (20)	100 (33)	$R_4OCH{=}\overset{+}{C}H\text{-}CH\text{-}NHR_2$
87 (100)	87 (100)	87 (100)	$R_3O\dot{C}H\text{-}\overset{+}{C}HNHR_2$

In order to try to resolve the structure of the C_5 acyl group, the three most probable isomers **9**, **10** and **11** were synthesised. The mass spectra of the permethylated derivatives **12** and **13** of compounds **9** and **10** respectively, were indistinguishable while that of **14** (from compound **11**) differed only in the presence of a weak ion at *m/z* 304 corresponding to the loss of C_2H_5 from $M^{+\cdot}$. Likewise, the mass spectra of the per-TMS derivatives **15**, **16**, **17** of isomers **9**, **10** and **11** respectively were all essentially identical to each other and to that of **18**, the TMS derivative of one of the major products of hydrolysis of wedeloside **1** itself. It was therefore apparent that mass spectrometry alone could not establish the structure of the C_5 acyl substituent on the aminosugar.

The two most common naturally-occuring hexoses in plant glycosides are glucose and galactose. For this reason it was expected that the aminoglycoside in wedeloside **1** would be a derivative of one of these. It had been reported[9] that 2 - acetylamino - 2 - deoxy - D - galactose and 2 - acetylamino - 2 - deoxy - D - glucose could be differentiated on the basis of differences in the relative intensities of certain fragment ions (e.g. *m/z* 233) in the mass spectra of their TMS derivatives. We therefore synthesised the TMS derivative of 2 - deoxy - 2 - (3 - methyl - 1 - oxobutyl) amino - D - galactose **23** and compared its mass spectrum with that of the isomeric glucose derivative **15** and that of the TMS hydrolysis product **18** of wedeloside. Although the differences between the spectra of the model compounds **15** and **23** were not as marked as had been observed for the acetylaminosugars,[9] the mass spectrum of **18** could be more closely aligned with that of the glucose derivative **15**. On this basis, we postulated that the glycoside in wedeloside was a derivative of 2-amino-2-deoxyglucose.







26: $R_1 = -O\text{-}[C_{20}H_{23}O_6(TMS)_4]$, $R_3 = R_4 = R_6 = TMS$, $R = -C_4H_9$
27: $R_1 = -O\text{-}[C_{20}H_{23}O_6(TMS)_4]$, $R_3 = R_3 = R_7$, $R_4 = R_6 = TMS$, $R = -C_4H_9$
28: $R_1 = \beta R_8$, $R_3 = R_4 = R_6 = H$, $R = -CH_2CH(CH_3)_2$
29: $R_1 = \beta R_8$, $R_3 = R_4 = R_6 = TMS$, $R = -CH_2CH(CH_3)_2$
30: $R_1 = \beta R_8$, $R_3 = R_7$, $R_4 = R_6 = H$, $R = CH_2CH(CH_3)_2$
31: $R_1 = \beta R_8$, $R_3 = R_7$, $R_4 = R_6 = TMS$, $R = CH_2CH(CH_3)_2$
32: $R_1 = OMe$, $R_2 = R_3 = R_4 = R_6 = H$, $R = -C_4H_9$
33: $R_1 = -O\text{-}[C_{20}H_{27}O_6]$, $R_3 = R_4 = R_6 = H$, $R = -C_4H_9$

Acid hydrolysis of wedeloside in aqueous methanol followed by trimethylsilylation of the resulting products gave four major peaks on the gc. By gc–MS, these were identified as: the aminoglucoside derivative **18** discussed above; its 1-Me analog **19**, whose mass spectrum compared well with that of the TMS derivative **21** of authentic methyl 2 - deoxy - 2 - (3 - methyl - 1 - oxobutyl)amino - α - D - glucopyranoside **20**; a mono-decarboxylated derivative **24** of the aglycone; and the mono-TMS derivative **25** of a compound which was tentatively identified as 3-phenylpropionic acid. This latter identification was confirmed by comparison of its mass spectrum and gc retention time with that of the TMS ester of authentic 3-phenylpropionic acid.

The 3-phenylpropionic acid identified on hydrolysis of underivatised wedeloside had not been observed as its methyl ester amongst the methanolysis products of the permethylated derivative **4**. The acid must therefore be ester linked to the toxin and cleaved from it by the action of the dimsyl anion in DMSO during the preparation of **4** in MeOH and its deuterium analog **5**. It was also found that the action of NaOMe on wedeloside selectively removed the 3-phenylpropionate ester, and the structure of the resultant product could be analysed as its TMS derivative **26**.

A partial mass spectrum of **26**, the silylated equivalent of **4**, is shown in Table 2. The ions selected arise in the main from the glycosidic portion of the compound and can be assigned[8, 10–12] to those segments of the sugar shown in Fig. 3. These ions are also present in the spectra of the silylated derivatives **15**, **21** and **29** of synthetic analogs of the glycoside (Table 2).

A comparison of the mass spectrum of **26**, the TMS derivative of the de-esterified compound from wedeloside, with that of the TMS derivative **27** of wedeloside itself clearly showed that the 3-phenylpropionate ester was on the glycosidic rather than the aglycone portion of the molecule. In particular, the ion at *m/z* 651 $[C_{20}H_{23}O_2(OTMS)_4]$ attributable to the aglycone, (*cf*, the *m/z* 419 ion in Fig. 2) is common to both mass spectra (Table 2). On the other hand, the ion at *m/z* 462 $(C_{20}H_{44}NO_5Si_3)$ in the spectrum of **26**, due to the glycoside ion *a*, shifts to *m/z* 522 $(C_{26}H_{44}NO_6Si_2)$ in the spectrum of TMS-wedeloside **27**. This corresponds to the replacement of —OTMS by $-OCO(CH_2)_2C_6H_5$.

The relative intensities of the other selected glycosidically-derived ions from **26** and **27** in Table 2 can be used to determine the position of linkage of the 3-phenylpropionate function to the sugar. Ions *h* and *g*, which contains C6 with its TMS substituent R_6 and C5 + C6 with part of the R_6 TMS group respectively, (Fig. 3) are common to the spectra of both **26** and **27**. This rules out C6 as the point of attachment of the ester. Similarly, the presence in these two mass spectra of ions *b* and *c*, which contain C3 and C4 plus the R_4 substituent but have lost R_3, indicates that the 3-phenylpropionate substituent is not on C4. This therefore leaves C3 as the only position remaining for linkage of the ester, given that the glycoside must be linked at C1 to the aglycone.

This is supported by the considerably reduced intensities of ions at *m/z* 215, 204 and 131 in the spectrum of **27** compared with that of **26**. In the latter, these ions correspond to structures *d*, *e* and *f* respectively, (Fig. 3)

Table 2. Partial mass spectra (*m/z* vs RI) and selected ion assignments for TMS derivatives **15**, **18**, **21**, **26**, **27**, **29**, **31**

	15	18	21	26	29	27	31	Ion
m/z \ m/z	551	551	493	1129	561	1189	621	$M^{+\cdot}$
651	-	-	-	9	-	22	-	Agl
522	-	-	-	-	-	35	0.8	*a*
462	-	-	0.2	22	0.2	-	-	*a*
268	7	5	14	18	5	8	4	*b*
288	5	4	5	24	16	100	100	*c*
217	22	21	14	42	21	42	31	
215	100	100	89	100	72	23[a]	8[a]	*d*
204	18	11	18	27	16	9[a]	-	*e*
191	10	6	6	19	9	11	6	
147	24	12	17	90	15	68	28	
131	99	99	100	27	100	15[a]	15[a]	*f*
117	12	11	11	22	10	12	7	*g*
103	11	6	7.5	26	22	34	32	*h*

[a] The residual ion intensities at these m/z values are not due to ions with structures as shown in column 9 and Figure 3.

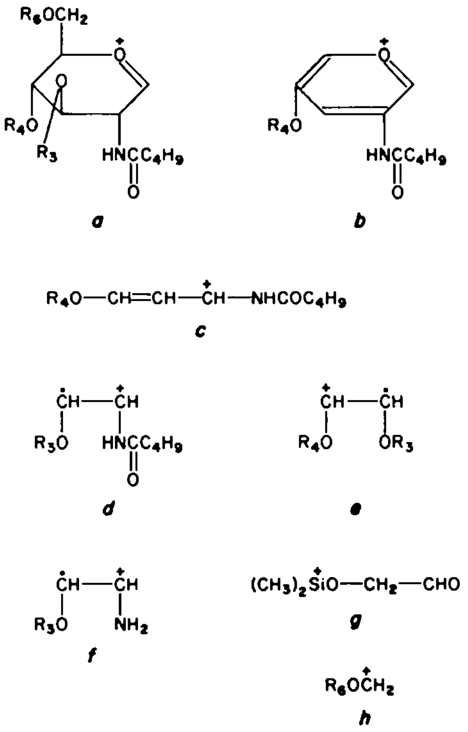


Fig. 3. Structures assigned[8,10–12] to selected ions in the mass spectra of comopunds in Table 2.

all of which have TMS as the R_3 substituent. That there are still residual ion intensities at these *m*/*z* values in the spectrum of **27** where $R_3 = CO(CH_2)_2Ph$ indicates that these must arise from ions other than *d*, *e* and *f*. There is a similar correlation between the mass spectra of the TMS derivatives **29** and **31** of the synthetic cyclohexyl glucopyranosides **28** and **30** which also differ only in the presence of a 3-phenylpropionyl ester on C3 of the latter compound (Table 2).

The above mass spectrometric studies on wedeloside allowed us to propose that the glycoside portion of **1**, which is C1 linked to the aglycone, is a 2 - deoxy - 3 - O - (1 - oxo - 3 - phenylpropyl) - 2 - valeramidoglucopyranoside.

By itself, the ^{13}C NMR spectrum of wedeloside could not be used to establish the undetermined structural features of the glycosidic portion of **1** due to the presence of unassigned resonances from the C_{20} aglycone moiety. Treatment of **1** with diazomethane to protect a β-dicarboxylic acid function in the aglycone also unexpectedly removed the phenylpropanoyl ester and subsequent acid-catalysed methanolysis of the product gave the methyl 2 - deoxy - 2 - valeramidoglucopyranoside **32**. The ^{13}C NMR spectrum of **32** was then compared with the spectra of the synthetic isomeric 2 - deoxy - 2 - valeramidoglucopyranosides **9**–**11**, which were analysed as anomeric mixtures.

The ^{13}C chemical shifts for the ring carbons in compounds **9**–**11**, which had been prepared to try to determine the structure of the C_5 N-acyl function by mass spectrometry, were assigned by reference to reported values[13] for 2 - acetylamino - 2 - deoxy - α - and β-D-glucopyranosides and by signal multiplicities (Table 3). Comparison of the chemical shift values for the remaining sidechain C atoms C8–C11 in these compounds clearly establishes the 3-methyl-1-oxobutyl structure **9** as the N-acyl grouping in **32** and therefore in wedeloside **1**. The ^{13}C NMR spectrum of **20**, the α-O-methyl derivative of **9**, was assigned by comparison with that reported for methyl 2 - acetylamino - 2 - deoxy - α - D - glucopyranoside[18] and is identical to that of the wedeloside product **32** (Table 3). This confirms the mass spectral assignment of a 2 - amino - 2 - deoxyglucopyranoside structure for the glycosidic portion of wedeloside. In addition, both **32** and **20** showed the same specific rotation and therefore the glucose-derived sugar in **1** must have the D absolute configuration. The glycosidic portion of **1** which still retained the ester functionality intact could not be isolated by hydrolysis of the parent compound. The preparation of the cyclohexyl analogue **30**, described below, and comparison of its ^{13}C NMR spectrum with that of **1**, allows confirmation of the proposed point of linkage of the ester in **1** and of the structure of the glycosyl moiety as a whole.

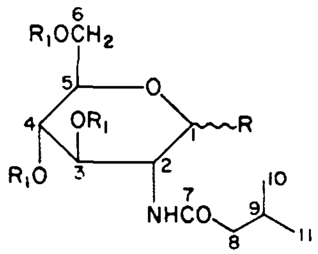
The anomeric mixture **9** was acetylated to give a mixture of the α- and β-anomers **34** and **35**, from which the latter could be separated by fractional crystallisation. In its ^{13}C NMR spectrum the skeletal C assignments for **35** (Table 4) could be made by reference to those reported for 2 - acetylamino - 1,3,4,6 - tetra - O - acetyl - 2 - deoxy - β - D - glucopyranose.[15] Conversion of **35** to the cyclohexyl derivative **37** via the pyranosyl bromide **36** was carried out under Koenigs–Knorr conditions using the method of Lemieux.[16] As expected, the ^{13}C signals for C1 and C2 were shifted strongly downfield in **37** compared to **35** while C3–C6 remained relatively unperturbed (Table 4).

Removal of the acetate protecting groups from **37** furnished compound **28**, which was converted into its benzylidene derivative **38** whose ^{13}C NMR spectral assignments (Table 4) were made by comparison with those of methyl 4,6 - O - benzylidene-β - D - glucopyranoside.[17] The signals for C3–C6 occur at similar chemical shift values in the two compounds while C1 and C2 in **38** are readily distinguished by their strong downfield and upfield shifts respectively.

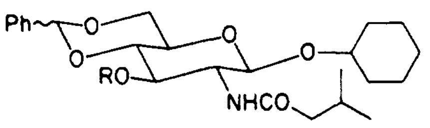
With all positions blocked in **38** with the exception of C3 it was simple to convert this compound into the 3-phenylpropionate derivative **39**. The ^{13}C NMR spectrum of **39** showed substantial upfield shifts of 5.0 and 2.8 ppm for C2 and C4 and a small downfield (0.9 ppm) shift for C3 compared to its precursor compound **38** (Table 4). Cleavage of the benzylidene protecting group with aqueous TFA gave the required model compound **30** with a cyclohexyl group in place of the diterpene aglycone in the structure **1** proposed for wedeloside. The ^{13}C chemical shift assignments for the glycosidic carbons in compounds **28** and **30** could be made by reference to the spectrum of the related methyl 2 - acetylamino - 2 - deoxy - β - D - glucopyranoside[14] and by the differences in shift values of C2, C3 and C4 in **28** and **30** compared with those observed for **38** and **39** above. These assignments together with those of wedeloside **1** and compound **33**, obtained from **1** by the removal of the 3-phenylpropanoyl moiety, are given in Table 5.

The chemical shift values for all the C atoms in the glycosidic portion of the de-esterified wedeloside derivative **33** were readily assigned by comparison with the spectrum of the synthetic cyclohexyl analog **28**.

These two sets of shift values are essentially identical with the exception of C1 (100.5 and 101.4 ppm) which reflects the difference in the two aglycone moieties (Table 5). From this, it can be deduced that **33** has the same β-anomeric configuration as **28** (*cf* **32** and **20** which are α-linked pyranosides) and therefore wedeloside **1** must also contain a β-anomeric sugar linkage. This is confirmed by the 8 Hz trans diaxial coupling constant



34: $R = \alpha - OAc, R_1 = Ac$
35: $R = \beta - OAc, R_1 = Ac$
36: $R = Br, R_1 = Ac$
37: $R = \beta - O - cC_6H_{11}, R_1 = Ac$



38: R = H
39: $R = COCH_2CH_2Ph$

Table 3. ^{13}C NMR assignments for synthetic compounds 9–11 and 20 and wedeloside derivative 32[a]

Carbon	9		10		11		32	20
	α	β	α	β	α	β		
1	91.3	95.4	91.4	95.6	91.4	95.4	98.7	98.7
2	54.4	57.4	54.4	56.9	54.4	57.0	54.0	54.0
3	72.0	74.3	72.0	74.3	72.0	74.2	71.4	71.4
4	71.0	70.6	70.9	70.6	71.0	70.6	70.6	70.6
5	70.6	76.4	70.6	76.3	70.6	76.4	72.2	72.1
6	61.2	61.2	61.1	61.1	61.6	61.2	61.1	61.0
7	177.3	177.3	181.3	181.3	178.0	178.0	177.4	177.3
8	45.3*t*	45.9*t*	42.8*d*	43.4*d*	36.0*t*	36.3*t*	45.3	45.3*t*
9	26.7*d*	26.7*d*	27.5*t*	27.5*t*	28.1*t*	28.1*t*	26.6	26.6*d*
10	22.0*q*	22.0*q*	11.5*q*	11.5*q*	22.0*t*	22.0*t*	22.0	22.0*q*
11	22.0*q*	22.0*q*	17.2*q*	17.2*q*	13.5*q*	13.5*q*	21.9	21.8*q*
-OMe	-	-	-	-	-	-	55.7	55.7

[a] All spectra were recorded at 15 MHz in D_2O using dioxane as standard. Chemical shift values are in ppm relative to TMS.

Table 4. ^{13}C NMR assignments for glucose carbons in synthetic intermediates[a]

Carbon	35	37	38	39
1	92.6	99.1	98.9	100.1
2	52.3	55.1	59.7	54.7
3	72.7	71.5	71.3	72.2
4	68.3	69.2	81.8	79.0
5	72.7	72.4	66.4	66.1
6	61.8	62.4	68.9	68.6

[a] All spectra were recorded at 15 MHz in $CDCl_3$ solution using TMS as standard. Chemical shift values are in ppm relative to TMS.

observed for the anomeric protons in the ^1H NMR spectra of 28 and 1 respectively.

There remained three signals in the sugar region of the spectrum of 33 at 73.5, 79.5 and 82.2 ppm which could only be ascribed to the aglycone. As expected, these three resonances remained virtually unchanged in the spectrum of the parent compound 1 whereas the six signals assigned to the glucose ring carbons all showed a change in chemical shift value due to the presence of the ester grouping. The individual assignments for C1 to C6 in wedeloside 1 were made initially by reference to compound 33 and confirmed by the close correlation with the values for the corresponding C atoms in the synthetic analog 30.

The conversion of 1 to 33 by the removal of the 3-phenylpropanoyl functionality produces a shift in each of the ^{13}C signals of the skeletal carbons of the glycosidic portion. The observed changes in chemical shift

Table 5. ^{13}C NMR assignments for glycosidic carbon atoms in wedeloside 1 and derivative 33 and synthetic cyclohexyl analogs 28 and 30[a]

Carbon	28	33	30	1
1	100.5	101.4	100.4	101.2
2	57.3	57.3	55.4	55.2
3	76.0	76.1	77.1	77.0
4	72.3	72.1	69.8	69.6
5	77.8	77.8	77.6	77.4
6	62.8	62.8	62.4	62.2
7	175.7	175.6	175.3	175.5
8	46.8	46.7	46.6	46.7
9	27.3	27.6	27.1	27.2
10	23.0	23.0	22.8	22.9
11	23.0	23.0	22.8	22.9
12			174.0	174.2
13			31.7	31.5
14			36.7	36.6
15			141.9	141.7
16,20			129.2	129.2
17,19			129.2	129.2
18			127.1	127.1
21	77.8		77.9	

[a] All spectra were recorded at 15 MHz in CD_3OD solution using TMS as standard. Chemical shift values are in ppm relative to TMS.

Table 6. Chemical shift differences Δδ (ppm) for C1–C6 on removal of the ester function at C3 in 30 and 1

	C1	C2	C3	C4	C5	C6
30 → 28	+0.1	+1.9	-1.1	+2.5	+0.2	+0.4
1 → 33	+0.2	+1.9	-0.9	+2.8	+0.4	+0.6

[a] Downfield shift is indicated by a positive value.

values Δδ (ppm) are listed in Table 6. Also listed in the Table are the corresponding differences in chemical shift values between the skeletal carbons of 30 and its analogue 28, which similarly lacks the 3-phenylpropanoyl functionality at C3. The correspondence between the Δδ values for the synthetic pair 30 and 28, and for 33 and 1 (Table 4) confirms the mass spectral conclusion that the 3-phenylpropionyl ester must be located at C3 in the glycosidic portion of 1.

The glycosidic portion of wedeloside 1 is therefore 2-deoxy-2-(3-methyl-1-oxobutyl)amino-3-O-(1-oxo-3-phenylpropyl)-D-glucopyranose, which is β-linked to the aglycone.

EXPERIMENTAL

2-Amino-2-deoxy-D-glucose hydrochloride was obtained from Sigma, 2-amino-2-deoxy-D-galactose from EGA-Chemie, 3-phenylpropanoic acid from L. Light and Co. Ltd., Trisil [$(Me_3Si)_2NH$: Me_3SiCl, 1:1] from Pierce and lactose from Ajax Chemicals.

Preparative tlc was carried out on Merk Silica gel 60 F_{254} or Aluminium oxide F_{254} (type T) pre-coated plates.

Gas chromatography was performed on a Varian 1400 using 2 m × 2 mm i.d. glass columns, with a N_2 carrier gas flow rate of 30 ml min^{-1}. The solid support was Gas Chrom Q (100–120 mesh). The mobile phase and temperature conditions varied and are described below in each case.

GC–MS (uncorrected) were measured on a Varian MAT 111 at 80 eV using gc conditions. Direct insertion mass spectra and high resolution accurate mass measurements were carried out on an AEI MS 902 at 70 eV. The reference compound used was heptacosafluorotributylamine. ^{13}C NMR were recorded on a JEOL JNM FX 60 at 15.04 MHz, ^1H NMR on a Varian HA 100 spectrometer and IR on a Perkin–Elmer model 257 spectrophotometer. All ^{13}C chemical shifts are in ppm related to TMS.

Wedeloside 1. The following is an improvement on the extraction procedure previously reported.[2] Toxicity levels were monitored at all stages of extraction and purification.

Milled air-dried leaf of *Wedelia asperrima* (500 g) was extracted three times with hot $MeOH/H_2O$ (1:1) and the extract concentrated under reduced pressure to a thick syrup. Excess H_2O was added and the H_2O insoluble material removed by centrifugation. The soln was then extracted with ether until a clear aqueous phase was obtained. This was concentrated to remove traces of ether, acidified to pH 1 with H_2SO_4 and then passed through a polyamide/celite column (100:200 g) made with water. After washing with water (4 l.) the toxin was displaced fromthe column with 0.1 N NH_4OH (2 l.) followed by water, until the eluate was neutral. The eluate was concentrated under reduced pressure (0.5 l.), acidified to pH 1 then extracted × 4 with BuOH (0.5 l.). The extract was washed once with water (0.5 l.), then the solvent removed under vacuum at 40° leaving a residue (5 g).

The residue (5 g) was dissolved in $AcOH/H_2O$ (70:30, 10 ml), High Flow Super Cell (15 g) added. The mixture was applied to a column (4 × 50 cm) containing High Flow Super Cell (30 g) which was prepared using the same solvent system slurried with toluene. The column was eluted with toluene (200 ml), $CHCl_3$ (200 ml) followed by increasing proportions of BuOH in $CHCl_3$ (each 200 ml). In every case the eluting solvent was saturated with one third of its volume of $AcOH/H_2O$ (70:30). Fractions (10 ml) containing the toxin were combined and dried under reduced pressure. The toxin was then purified by ascending chromatography on a silica gel G column with $CHCl_3$:MeOH:AcOH:H_2O (65:25:5:5) using a technique described previously,[18] to yield pure wedelia toxin (100 mg) as an amorphous powder, m.p. 168–170°. IR ν_{max}, mull, (KBr) 3360 (OH), 1706 (carboxyl C=O), 1634 (amide C=O) cm^{-1}. UV λ_{max} (MeOH) 222, 258 nm. $[\alpha]_D^{25} - 52°$ (c = 2.5, CH_3OH). ^{13}C NMR (CD_3OD); for glycosidic carbons see Table 3, 160.4, 108.9, 82.2, 79.5, 73.5, 59.0, 53.5, 49.3, 48.2, 46.7, 43.9, 41.0, 40.0, 35.8, 24.0, 20.9, 17.7. ^1H NMR [DMSO (d_6) − D_2O] δ 4.55 (d, J = 8 Hz, anomeric βH).

Preparation of 4 *and* 5. Two separate samples of 1 (2 mg) were taken up in DMSO (0.5 ml) and treated under ultrasonification with dimsyl sodium in DMSO (2N, 1 ml) for 1 h. The resultant suspensions were separately treated with CH_3I (1 ml) and CD_3I (1 ml) at 0° and then allowed to stand overnight at r.t. Excess dimsyl anion was destroyed by careful addition of MeOH. The residue was diluted with H_2O and extracted with $CHCl_3$. The organic layer was washed with H_2O, dried and concentrated. The required products were purified by plc on silica (30% CH_3CN: C_6H_6, R_f 0.25) yielding 1.6 mg of each. 4 (For mass spectrum see Fig. 1a). $M^{+\cdot}$ 737.4334, $C_{39}H_{63}NO_{12}$ calc. 737.4350; 521.2730, $C_{31}H_{39}NO_6$ calc. 521.2777; 419.2426, $C_{24}H_{35}H_6$ calc. 419.2433; 270.1697, $C_{14}H_{24}NO_4$ calc. 270.1705; 184.1333, $C_{10}H_{18}NO_2$ calc. 184.1338; 87.0691, C_4H_9NO calc. 87.0684. 5 (For mass spectrum see Fig. 1b).

Acid methanolysis of 4. Compound 4 (0.5 mg) was heated at 60° in dry MeOH (1 ml) over Dowex 50 W resin (H^+ form) for 2 hr. An aliquot was subjected directly to gc–MS (2% SE30; 100–200°, Δ 10° min^{-1}). Two compounds 6 and 7 were observed. 6 (R_t 10.6 min). MS m/z (rel. int.): $M^{+\cdot}$ 333(0.1), 318(0.4), 302(0.4), 301(0.4), 288(0.2), 286(0.9), 270(0.3), 259(0.3), 256(0.6), 254(0.7), 242(0.5), 224(9), 218(1.5), 186(4), 185(4), 184(33), 172(2.5), 171(5), 141(7), 140(7), 126(3), 118(2.5), 117(38), 116(2.5), 115(6), 111(3), 102(17), 101(14), 100(20), 88(18), 87(100). 7 (R_t 18.7 min). MS m/z (rel. int.): $M^{+\cdot}$ 436(3), [measured 436.2463; $C_{24}H_{36}O_7$, calc. 436.2460], 404(8), 165(46), 152(18), 145(10), 129(15), 128(100), 113(18), 105(13), 97(46), 91(15).

2-*Deoxy*-2-(3-*methyl*-1-*oxobutyl*)*amino*-*D*-*glucopyranose* 9. 2-Amino-2-deoxy-D-glucose hydrochloride (1 g) in dry pyridine (20 ml) was treated with Trisil (4 ml) under N_2. After 2 h at r.t. the mixture was cooled to 0° and 3-methylbutanoyl chloride (1.5 ml) was added dropwise. After 12 h at r.t. the mixture was added to pre-cooled-Claq (200 ml, 2N) and rapidly extracted with ether (200 ml). The ether layer was evaporated to dryness and the residue was stirred in aqueous THF (90%) for 2 weeks. The resultant soln was evaporated to dryness, triturated with ether and the resultant solid crystallised from H_2O to yield the required 9 (0.7 g, 57%) m.p. 208–210°. (Found: C, 49.92; H, 7.96; N, 5.32. $C_{11}H_{21}NO_6$ requires: C, 50.18; H, 8.04; N, 5.32%). ^{13}C NMR (D_2O), standard dioxane; β: 177.3, 95.4, 76.4, 74.3, 71.0, 61.2, 57.0, 45.9, 26.7, 22.0, 22.0. α: 177.3, 91.3, 72.0, 71.0, 69.6, 61.2, 54.4, 45.3, 26.7, 22.0, 22.0 ppm.

Methyl 2-*deoxy*-*N*-*methyl*-3,4,6-*tri*-O-*methyl*-2-(3-*methyl*-1-*oxobutyl*)*amino*-β-*D*-*glucopyranoside* 12. 9 (100 mg) in DMF (5 ml) was treated with NaH (300 mg). The suspension was ultrasonicated for 0.5 hr, cooled to 0° and MeI (3.5 ml) was added dropwise. Excess hydride was destroyed by addition of MeOH. The resultant soln was partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ layer was washed with H_2O, dried and evaporated. The residue was subjected to plc on silica [CH_3CN: C_6H_6, 1:1] and the title compound **12** obtained as an oil (R_f 0.5). (Found: C, 57.99; H, 9.36; N, 4.16, $C_{16}H_{31}NO_6$ requires: C, 57.66; H, 9.36; N, 4.21%). ^1H NMR ($CECl_3$) δ 4.30 (d, J = 7.5 Hz, anomeric β H). ^{13}C NMR (C_6D_6) standard TMS; 172.8, 172.2, 82.0, 81.6, 80.7, 75.1, 71.5, 62.2, 60.1, 5908, 56.4, 43.6, 42.3, 27.7, 25.3, 22.9, 22.7 ppm. gc–MS: (2% OV-17; 150–300°, Δ10° min^{-1}; R_t 7.8 *min*). MS *m/z* (Rel. int.): $M^{+\cdot}$ 333(0.1), 318(0.4), 302(0.3), 301(0.4), 288(0.4), 286(3), 270(0.5), 259(0.5, 256(0.7), 254(1), 242(0.5), 224(8), 218(1.5), 186(4), 185(5), 184(33), 172(3), 171(8), 141(7), 140(11), 126(3.5), 118(3), 117(46), 116(2.8), 115(9.5), 111(2.7), 102(28), 101(19), 100(33), 88(23), 87(100).

2 - *Deoxy* - 2 - (2 - *methyl* - 1 - *oxobutyl)amino* - D - *glucopyranose* **10**. This was prepared from 2 - amino - 2 - deoxy - D - glucose hydrochloride and 2-methylbutanoyl chloride by the same method as **9**, except that the intermediate persilyl derivative was hydrolysed in 0.1 N HCl for 24 hr, yield (60%). m.p. 214°. (Found: C, 50.01; H, 8.08; N, 5.28. $C_{11}H_{21}NO_6$ requires: C, 50.18; H, 8.04; N, 5.32%). ^{13}C NMR (D_2O) standard dioxane; β: 181.3, 95.6, 76.3, 74.3, 69.9, 61.1, 56.9, 43.4, 27.5, 17.2, 11.5. α: 181.3, 91.4, 72.0, 69.9, 69.6, 54.4, 42.8, 27.5, 17.2, 11.5 ppm.

Methyl 2 - *deoxy* - N - *methyl* - 3,4,6 - *tri* - O - *methyl* - 2 - (2 - *methyl* - 1 - *oxobutyl)amino* - β - D - *glucopyranoside* **13**, was prepared from **10** by the same method as was **12** from **9**. ^1H NMR ($CDCl_3$) δ 4.30 (d, J = 8 Hz, anomeric βH). Gc–MS (2% OV-17, 150–300°, Δ10° min^{-1}; R_t 7.8 min). MS *m/z* (rel. int.): $M^{+\cdot}$ 333(0.2), 318(0.3), 302(0.5), 301(0.3), 288(0.7), 270(0.5), 259(1.2), 256(1.5), 254(0.5), 242(0.5), 224(11), 218(9), 186(9), 185(6), 184(46), 172(5), 171(15), 141(13), 140(11), 126(2.5), 118(5), 117(64), 116(2), 115(6), 111(2.7), 102(43), 101(27), 100(29), 80(21), 87(100).

2 - *Deoxy* - 2 - (1 - *oxopentyl)amino* - D - *glucopyranose* **11**. This was prepared by the same method as **9** from 2 - amino - 2 - deoxy - D - glucose hydrochloride and pentanoyl chloride, yield (55%). m.p. 200–202°. (Found: C, 50.11; H, 8.07; N, 5.21. $C_{11}H_{21}NO_6$ requires: C, 50.18; H, 8.04; N, 5.32%). ^{13}C NMR (D_2O) standard dioxane; β: 178.0, 95.4, 76.4, 74.2, 70.6, 61.2, 57.0, 36.3, 28.1, 22.0, 13.5. α: 178.0, 91.2, 71.9, 71.0, 70.6, 54.4, 36.0, 28.1, 22.0, 13.5 ppm.

Methyl 2 - *deoxy* - N - *methyl* - 3,4,6 - *tri* - O - *methyl* - 2 - (1 - *oxopentyl)amino* - β - D - *glucopyranoside* **14** was prepared from **11** as were **12** and **13** from **9** and **10** respectively. ^1H NMR ($CDCl_3$) δ 4.30 (d, J = 8 Hz, anomeric β H). Gc–MS (2% OV-17; 150–300°, Δ 10° min^{-1}; R_t 8.0 min). MS *m/z* (rel. int.): $M^{+\cdot}$ 333(0.3), 318(0.3), 304(1.7), 302(0.8), 301(0.5), 288(0.7), 270(0.5), 259(2.0), 256(2.0), 254(0.5), 242(1), 224(18), 218(11), 186(9), 185(12), 184(52), 172(6), 171(16), 141(8), 140(11), 126(4), 118(5.0), 117(57), 116(4), 115(10), 111(3), 102(41), 101(23), 100(26), 88(23), 87(100).

Preparation of **15**, **16** *and* **17**, *the silyl derivatives of* **9**, **10** and **11** *respectively*. These derivatives were prepared from 2 - amino - 2 - deoxy - D - glucose hydrochloride (1 mg) in Trisil: pyridine (3:5, 1 ml) by the action of the appropriate acid chloride (10 μl) and also by direct silylation of **9**, **10** and **11**. The products were directly examined by gc–MS (2% OV-17; 130–290°, Δ 10° min^{-1}; R_T 10.0 min). Their mass spectra were essentially identical, typified by that of **15** *m/z* (rel. int.): 537(1.0), 536(2.4), 448(0.5), 446(1.5), 358(0.8), 356(4), 305(4), 303(1.8), 302(4.8), 301(8.5), 288(2.7), 286(0.1), 272(2.4), 268(7), 266(2.5), 233(8), 228(5), 218(9), 217(22), 216(21), 215(100), 204(18), 191(10), 147(24), 132(15), 131(99), 117(12), 103(11).

Hydrolysis of wedeloside **1**. **1** (1 mg) was heated at 90° for 2 h in MeOH/HCl/H_2O (1 ml; 1:0.5 (4N): 0.5) and evaporated to dryness in *vacuo*. A sample was silylated with Trisil and examined by gc–MS (2% OV-17; 100–290°, Δ 10° min^{-1}). Four major compounds were observed. **25**. R_t 17.8 min: MS *m/z* (rel. int. > 20%): 624(14), 281(72), 244(63), 230(23), 229(100).

18 R_t 13.4 min: MS *m/z* (rel. int.): 537(0.5), 536(1.4), 448(0.5), 446(0.9), 358(0.8), 356(2.2), 305(4), 303(1.5), 302(4.4), 301(6.2), 288(1.6), 286(0.7), 272(1.8), 268(5), 266(5), 233(10), 228(4), 218(8), 217(21), 216(12), 215(100), 204(11), 191(6), 147(12), 132(8), 131(99), 117(11), 103(6).

19 R_t 12.9 min: MS *m/z* (rel. int.): 478(0.1), 462(0.05), 446(0.1), 356(1.2), 301(2), 268(7), 217(6), 216(7), 215(42), 204(8), 178(5), 147(15), 144(6), 131(81), 129(4), 117(7), 103(7), 89(4), 85(5), 79(10), 75(30), 74(10), 73(100).

24 R_t 4.8 min: MS *m/z* (rel. int.): 223(1), 207(13), 189(2), 147(9). 132(4), 131(5), 105(6), 104(80), 91(19), 79(19), 78(5), 77(10), 76(5), 75(100), 73(48), 52(11), 51(10). The mass spectrum of **24** was essentially the same as that of the TMS ester of authentic 3-phenylpropanoic acid.

Methyl 2 - *deoxy* - 2 - (3 - *methyl* - 1 - *oxobutyl)amino* - α - D - *glucopyranoside* **20**. **9** (200 mg) was refluxed in MeOH over Dowex 50W resin (H^+ form) for 2 hr. The solid obtained after filtration and evaporation was crystallised from MeCN yielding the title compound (65%), m.p. 198–203°. (Found: C, 51.82; H, 8.20; N, 5.23. $C_{12}H_{23}NO_6$ requires: C, 51.98; H, 8.30; N, 5.04%). ^1H NMR [DMSO(d_6)] δ 4.57 (d, J = 3.5 Hz, anomeric α H). ^{13}C NMR (D_2O) standard dioxane; 177.3, 98.7, 72.1, 71.4, 70.6, 61.0, 55.7, 54.0, 45.3, 26.6, 22.0, 21.8. $[\alpha]_D^{25}$ +91° (*c* 0.73, MeOH). *On silylation* **20** yields **21**. gc–MS (2% OV-17; 210°; R_t 3.4 min). MS *m/z* (rel. int.): 478(1), 462(0.2), 446(0.8), 372(1), 356(4), 301(5), 268(14), 217(14), 216(21), 215(69), 204(18), 191(5), 178(8), 147(17), 132(13), 131(100), 129(5), 117(11), 103(8), 89(5), 85(5), 79(7), 75(31), 74(8), 73(85).

Methyl 4 - *deoxy* - N - *methyl* - 2,3,6 - *tri* - O - *methyl* - 4 - (3 - *methyl* - 1 - *oxobutyl)amino* - α - D - *galactopyranoside* **22**. This compound was prepared from permethylated lactose via acid methanolysis, tosylation of the 4-OH group on the glucoside, azide displacement followed by reduction to the amine, amide formation and N-methylation. A full description of the synthetic procedures will be published elsewhere. m.p. 77–78°. IR ν_{max} 1631 cm^{-1}. (Found: C, 57.35; H, 9.11; N, 3.96. $C_{16}H_{31}NO_6$ requires: C, 57.65; H, 9.30; N, 4.20%). ^1H NMR ($CDCl_3$): δ 5.4 (bm, 1H), 4.97 (d, J = 5 Hz, 1H), 4.4-3.6 (bm, 5H), 3.55 (s, 3H), 3.47 (s, 3H), 3.40 (s, 3H), 3.40 (s, 1.3 H), 3.18 (s, 1.7 H), 2.25 (d, J = 3 Hz, 2H), 2.8–2.4 (bm, 1H), 1.0 (d, 6H). MS *m/z* (rel. int.): $M^{+\cdot}$ 333(5), 318(10), 303(10), 302(56), 301(63), 286(5), 270(10), 258(10), 256(15), 242(49), 241(46), 228(29), 216(13), 197(16), 196(100), 186(16), 185(19), 184(86), 173(19), 171(26), 158(60), 140(69), 131(19), 126(19), 116(15), 112(10), 101(76), 100(69), 88(86), 87(73).

TMS derivative of 2 - *deoxy* - 2 - (3 - *methyl* - 1 - *oxobutyl)amino* - D - *galactose* **23**. 2 - Amino - 2 - deoxy - D - galactose hydrochloride (1 mg) in Trisil: pyridine (3:5, 1 ml) was treated with 3-methylbutanoyl chloride (10 μl) and directly examined by gc-MS (2% OV-17; 150–250°, Δ 10° min^{-1}; R_t 7.8 min). MS *m/z* (rel. int.): 537(3), 536(3.5), 464(1), 462(1.5), 450(1), 448(1), 446(1.5), 359(2), 358(1.5), 357(2.5), 356(4.5), 306(2), 305(6), 302(3), 301(4.5), 288(3), 272(4.5), 268(4), 266(4), 233(15), 228(8), 218(10), 217(21), 216(14), 215(85), 204(18), 191(9), 147(29), 132(11), 131(100), 117(10), 103(10).

Preparation of **26**. **1** (5 mg) was added to anhyd MeOH to which a catalytic amount of Na had been added. After 48 h at r.t. the solution was acidified by the addition of Dowex 50W resin (H^+ form) filtered, and evaporated to dryness. A sample of the resultant solid was suspended in CH_3CN and silylated with BSTFA/TMCS (9:1) to yield **26**. MS *m/z* (rel.int.): $M^{+\cdot}$ 1129(0.8), 1115(2.5), 1114(3), 997(1), 957(1.2), 811(1.4), 651(9), 535(5), 462(22), 433(9), 372(40), 343(7), 301(12), 300(5), 299(15), 289(9), 288(24), 282(8), 281(15), 269(7), 268(18), 245(10), 244(27), 230(12), 229 (40), 228(24), 219(10), 218(30), 217(42), 216(23), 215(100), 205(13), 204(27), 193(8), 192(11), 191(19), 169(8), 168(12), 149(16), 148(15), 147(90), 143(13), 133(14), 131(27), 129(19), 126(17), 117(22), 103(26).

The silyl derivative **27** *of wedeloside*. **1** (0.5 mg) was suspended in MeCN (10 μl) and silylated with BSTFA/TMCS [(9:1), 50 μl] to give **27**. An aliquot (5 μl) was subjected to MS *m/z* (rel.int.): $M^{+\cdot}$ 1189(2), 1175(3), 1174(4), 1102(2), 1099(2), 1074(0.9), 1073(0.8), 1019(3), 1018(3), 652(13), 651(22), 535(12), 533(5), 523(15), 522(35), 462(7), 445(14), 443(15), 373(15), 372(45), 343(12), 301(15), 300(47), 289(7), 288(17), 282(12), 281(15), 268(8), 229(46), 228(100), 218(22), 217(42), 216(6), 215(23), 207(10), 205(7), 204(9), 199(7), 198(12), 191(11), 169(14), 157(12), 148(9), 147(68), 144(14), 143(30), 138(10), 133(16), 131(15), 129(16), 126(12), 117(12), 105(24), 104(15), 103(34).

Preparation of **29** *the silylated derivative of* **28**. Compound **28** (0.5 mg) was suspended in MeCN (10 μl) and silylated with BSTFA/TMCS [(9:1), 50 μl] to give **29**. An aliquot (1 μl) was subjected to gc–MS, (2% OV-17; 150–300°, Δ 10° min^{-1}; *R*$_t$ 10.5 min). MS *m/z* (rel. int.): 546(0.5), 478(0.2), 462(0.2), 458(0.3), 446(0.3), 373(1.8), 372(2.5), 356(1.2), 315(1.4), 302(1.5), 301(1), 288(4), 268(5), 244(2), 243(5), 229(2), 228(16), 218(11), 217(21), 216(17), 215(72), 204(16), 192(1.5), 191(7), 173(4), 172(3), 170(4), 169(10), 161(9), 158(6), 157(4), 147(15), 145(4), 144(22), 143(8), 133(9), 132(17), 131(100), 129(10), 126(6.5), 117(10), 116(7), 104(2), 103(22).

Preparation of **31** *the silylated derivative of* **30**. Compound **30** was silylated by the same method as above to yield **31**, and its gc–MS recorded (2% OV-17; 250–310°, Δ 10° min^{-1}; *R*$_t$ 7 min). MS *m/z* (rel. int.): $M^{+\cdot}$ 621(0.2), 606(0.8), 522(0.8), 521(1), 374(2), 372(3.8), 370(1.8), 348(3), 343(3), 310(7), 289(3.5), 288(3), 268(4), 229(20), 228(100), 218(15), 217(31), 216(5), 215(8), 199(4), 198(5), 191(6), 171(3), 170(12), 169(13), 156(8), 147(28), 145(8), 144(52), 143(30), 133(24), 132(10), 131(15), 130(3), 129(12), 126(21), 117(7), 113(10), 105(31), 104(12), 103(32).

Preparation of **32**. **1** (37 mg) was taken up in MeOH and treated with excess etheral diazomethane for 1 hr. The soln was evaporated to dryness *in vacuo* and the residue was triturated with ether yielding a solid (30 mg), which was then heated in MeOH over Dowex 50W resin (H^+ form) at 60° for 4 hr. The residue after filtration and evaporation, was partitioned between H_2O and $CHCl_3$. The aqueous phase was repeatedly extracted with $CHCl_3$ to remove aglycone related material. Evaporation of the aqueous phase yielded **32** (12 mg) which was recrystallised from CH_3CN (4 mg). $[\alpha]_D^{25} + 92°$ ($c = 0.2$, MeOH). ^{13}C NMR; see Table 1. A sample was silylated GC–MS (2% OV-17; 210°; R$_t$ 3.4 min) MS *m/z* (rel. int.): 478(1), 462(0.2), 446(0.5), 372(0.5), 356(2), 301(3), 268(8), 217(15), 216(9), 215(55), 204(10), 191(5), 178(7), 147(27), 132(13), 131(100).

Preparation of **33**. **1** (68 mg) was added to anhyd MeOH to which a small pellet of Na had been added. After 48 hr at r.t. the solution was acidified by the addition of Dowex 50W resin (H^+ form), filtered and evaporated to dryness. The residue was well triturated with ether to remove methyl 3-phenylpropanoate yielding solid **33** (54 mg). ^{13}C NMR (CD_3OD); for glycosidic carbons see Table 3. 160.7, 108.7, 82.2, 79.6, 73.2, 59.1, 53.8, 49.9, 48.3, 46.9, 44.0, 40.1, 35.9, 24.2, 20.9, 17.7. For the MS of the TMS derivative see **26**.

1,3,4,6 - *Tetra* - O - *acetyl* - 2 - *deoxy* - 2 - (3 - *methyl* - 1 - *oxobutyl*)*amino* - *β* - D - *glucopyranose* **35**. The anomeric mixture **9** (300 mg) was added to a pre-cooled mixture of AC_2O (4.2 g) in pyridine (5.4 g) and stirred overnight at r.t. The mixture was poured into iced H_2O (100 ml) with vigorous stirring and then extracted with $CHCl_3$. The $CHCl_3$ layer was washed with HClaq and sat $NaHCO_3$aq, dried over $MgSO_4$, and evaporated to dryness yielding a crude mixture of **34** and **35**. Crystallisation from EtOH yielded **35** (40%) m.p. 156–158°. (Found: C, 53.01; H, 6.99; N, 3.10. $C_{19}H_{29}NO_{10}$ requires: C, 52.90; H, 6.72; n, 3.25%). ^1H NMR ($CDCl_3$, D_2O shake) δ 5.7 (d, $J = 8.5$ Hz, 1H), 4.85 (bm, 2H), 4.70–3.75 (4H), 2.1-2 (2H), 2.04, 2.09 (4 × OAc), 1.25 (m, 1H), 0.9 (d, $J = 5$ Hz, 6H). ^{13}C NMR ($CDCl_3$); for Cl–C6 see Table 2; 172.4, 171.1, 170.6, 169.3, 46.0, 26.1, 22.2, 20.7. MS *m/z* (rel. int.): 389(1), 388(3), 373(1), 372(4), 371(3), 360(2), 330(8), 329(34), 312(5), 283(22), 209(40), 208(24), 197(21), 195(28), 178(21), 168(34), 167(100), 166(30), 156(62), 138(21), 126(73), 125(40), 114(21), 97(27), 85(74), 83(52), 72(29), 60(27), 57(92).

Cyclohexyl 3,4,6 - *tri* - O - *acetyl* - 2 - *deoxy* - 2 - (3 - *methyl* - 1 - *oxobutyl*)*amino* - *β* - D - *glucopyranoside* **37**. Mercuric cyanide (2.47 g, 9.8 mmol), anhy $CaSO_4$ (4.5 g), and molecular sieve 4A were added to a soln of cyclohexanol (1 g, 10 mmol) in dry C_6H_6 (50 ml). The mixture was stirred at r.t. for 1 hr before the addition of **36** (2 g, 44 mmol) prepared from **35** by the method of Lloyd and Stracey.[19] The mixture was stirred for 5 days, and then diluted with $CHCl_3$ (200 ml). The solids were removed by filtration and the $CHCl_3$ removed *in vacuo*. The resultant solid was crystallised from ether, triturated with hot pet ether, hot H_2O and then recrystallised from ether yielding **37** (800 mg, 38%) m.p. 200° (Found: C, 58.32; H, 7.79; N, 2.82. $C_{23}H_{37}NO_9$ requires: C, 58.60; H, 7.85; N, 2.97%). ^{13}C NMR ($CDCl_3$); for Cl–C6, see Table 4, 172.4, 170.6, 169.5, 77.8, 46.1, 33.4, 31.8, 25.9, 25.6, 23.8, 22.3, 20.7. MS *m/z* (rel. int.): 456(1), 390(8), 370(40), 329(10), 311(11), 288(10), 284(14), 283(100), 270(10), 244(15), 241(16), 240(10), 229(10), 228(40), 223(18), 199(16), 198(59), 186(10), 185(11), 182(10), 181(22), 168(21), 157(11), 156(85), 155(30), 143(38), 139(10), 138(15), 126(35), 114(20), 113(10), 97(16), 96(15), 85(40), 83(15), 72(20).

Cyclohexyl 2 - *deoxy* - 2 - (3 - *methyl* - 1 - *oxobutyl*)*amino* - *β* - D - *glucopyranoside* **28**. **37** (780 *mg*) was added to anhyd MeOH (5 ml), which had been pretreated with a catalytic amount of Na. After 48 hr at r.t. the seoln was neutralised with Dowex 50W resin (H^+ form), which was thenremoved by filtration. The soln was evaporated to dryness yielding a colourless solid, which was crystallised from EtOAc: $CHCl_3$ to give **28** (394 mg, 69%), m.p. 214–216°. (Found: C, 58.84; H, 9.00; N, 4.15. $C_{17}H_{31}NO_6$ requires: C, 59.13; H, 8.98; N, 4.05%). ^{13}C NMR (CD_3OD); for Cl–Cll see Table 4, 34.5, 32.7, 26.7, 24.9. MS *m/z* (rel. int.): 314(2), 296(1), 272(2), 262(2), 254(40), 244(5), 227(15), 200(3), 199(7), 190(4), 181(5), 178(8), 173(5), 172(50), 162(12), 157(22), 156(100), 144(15), 143(20), 142(5), 126(12), 115(5), 114(7), 102(15), 98(5), 97(7), 96(5), 89(22), 88(8), 87(5), 85(51), 83(14), 74(12), 73(9), 72(67).

Cyclohexyl 4,6 - O - *benzylidene* - 2 - *deoxy* - 2 - (3 - *methyl* - 1 - *oxobutyl*)*amino* - *β* - D - *glucopyranoside* **38**. **37** (470 mg) was taken up in DMF (5.5 ml) and treated with *α,α*-dimethyoxytoluene[20] (3.5 ml) and a catalytic amount of *p*-toluenesulphonic acid (25 mg). The mixture was heated at 40° for 1.5 hr, at 40° for 0.5 hr *in vacuo*, and finally at 40° for a further vacuum and the resultant solid washed with dil $NaHCO_3$aq, H_2O, and pet.ether. Preparative tlc on silica gel (20% MeCN:$CHCl_3$) (r.f.0.25) yielded a white solid which on crystallisation from C_6H_6 afforded **38** (46%) m.p. 247°. (Found: C, 66.45; H, 8.07; N, 3.31. $C_{24}H_{35}NO_6$ requires: C, 66.51; H, 8.08; N, 3.23%). In ^1H NMR ($CDCl_3$) 4.92 δ (d, $J = 8$ Hz, 1H). ^{13}C NMR ($CDCl_3$); for C1–C6 see Table 4, 174.3, 137.4, 129.2, 128.4, 126.4, 102.0, 46.3, 33.6, 31.9, 26.4, 25.5, 24.0, 22.4. ^{13}C NMR (CD_3OD); 175.9, 139.3, 129.9, 129.2, 127.6, 103.0, 101.6, 83.3, 78.5, 72.7, 69.9, 67.7, 58.3, 34.7, 32.8, 27.4, 26.7, 24.9, 23.0. MS *m/z* (rel. int.): M^+ 433(1), 432(2), 350(14), 334(7), 333(15), 332(36), 291(5), 266(15), 251(18), 250(66), 244(26), 232(5), 227(10), 207(5), 183(5), 181(13), 162(9), 160(7), 157(10), 156(100), 155(22), 149(7), 144(6), 143(28), 126(11), 114(6), 107(32), 106(10), 105(57), 102(13), 101(22), 100(6), 91(8), 86(5), 85(48), 83(15), 72(26).

Cyclohexyl 4,6, - O - *benzylidene* - 2 - *deoxy* - (3 - *methyl* - 1 - *oxobutyl*)*amino* - 3 - O - (1 - *oxo* - 3 - *phenylpropyl*) - *β* - D - *glucopyranoside* **39**. **38** (410 mg) was taken up in pyridine (40 ml), cooled to 0° and treated, by dropwise addition, with 3-phenylpropanoic anhydride (540 mg). After 20 hr at r.t. the mixture was added, with stirring, to iced-water (200 ml). The resultant suspension was extracted with $CHCl_3$ and the $CHCl_3$ layer successively washed with 4N HCl, H_2O, sat $NaHCO_3$aq and H_2O. After drying ($MgSO_4$), the $CHCl_3$ was removed *in vacuo* and the resultant solid crystallised from C_6H_6: pet. ether (60–80) yielding **39** (360 mg, 67%), m.p. 242–244°. (Found: C, 70.10; H, 7.65; N, 2.30. $C_{33}H_{43}NO_7$ requires: C, 70.08; H, 7.61; N, 2.47%). $M^{+\cdot}$ found: 565.3040, requires 565.3039. ^{13}C NMR ($CDCl_3$); for Cl–C6 see Table 4, 173.1, 172.2, 140.0, 137.1, 128.8, 128.0, 126.2, 125.9, 101.2, 77.2, 46.1, 33.2, 31.5, 30.6, 25.8, 25.4, 23.7, 22.5. MS *m/z* (rel. int.): 565(26), 482(10), 466(10), 465(23), 464(41), 382(11), 376(27), 333(11), 288(25), 157(10), 156(100), 155(50), 149(24), 143(20), 133(45), 127(25), 126(33), 107(35), 106(10), 104(15), 101(27), 98(14), 91(70), 85(40), 83(14), 78(12), 72(40).

Cyclohexyl 2 - *deoxy* - 2 - (3 - *methyl* - 1 - *oxobutyl*)*amino* - 3 - O - (1 - *oxo* - 3 - *phenylpropyl*) - *β* - D - *glucopyranoside* **30**. **39** (140 mg) was added to 90% aq. TFA at −4°. The suspension was stirred for 2 hr and then the solvent was removed under high vacuum and by co-distillation with MeOH. The resultant solid was purified by plc on silica using EtOAc (r.f. 0.6). Subsequent crystallisation of the resultant solid from CH_2Cl_2: pet ether yielded **30**, (50%). m.p. 115–116°. (Found: C, 65.36; H, 8.26; N, 3.25. $C_{26}H_{39}NO_7$ requires: C, 65.40; H, 8.17; N, 2.94%). ^{13}C NMR

(CD_3OD); for C1–C21 see Table 6, 34.4, 32.6, 26.7, 24.8. MS m/z (rel. int.): 477(0.1), 377(2.5), 254(32), 227(10), 199(5), 178(8), 172(45), 162(10), 157(20), 156(100), 144(15), 143(21), 126(14), 102(15), 97(10), 91(6), 89(20), 85(50), 83(14), 72(71).

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REFERENCES

[1] P. B. Oelrichs and W. A. Müller, *Toxicon* **10**, 63 (1972); and refs therein.
[2] P. B. Oelrichs, P. J. Vallely, J. K. MacLeod and I. A. S. Lewis, *Lloydia*, **43**, 414 (1980).
[3] T. Ajello, F. Piozzi, A. Quilico and V. Spiro, *Gazz. Chim. Ital.* **93**, 867 (1963); F. Piozzi, A. Quilico, R. Mondelli, T. Ajello, V. Spiro and A. Melera, *Tetrahedron* Suppl. 8, Part 11, 515 (1966); F. Piozzi, A. Quilico, C. Fuganti, T. Ajello and V. Spiro, *Gazz. Chim. Ital.* **97**, 935 (1967).
[4] B. Danieli, E. Bombardelli, A. Bonati and B. Gabetta, *Phytochemistry* **11**, 3501 (1972); E. Bombardelli, *Atractyloside: chemistry, biochemistry and toxicology* (Edited R. Santi, and S. Luciani), pp. 33–38. Piccin Medical Books, Padova (1978).
[5] G. Defaye, P. M. Vignais and P. V. Vignais, *C. R. Acad. Sci. Paris* **273**, 2671 (1971); G. Defaye, D. Horton and J. D. Wander, *Bull. Soc. Chim. Fr.* 615 (1973).
[6] H. Oberman and G. Spiteller, *Chem. Ber.* **109**, 3450 (1976); H. Richter and G. Spiteller, *Ibid.* **111**, 3506 (1978).
[7] S. Hakomori, *J. Biochem.* **55**, 205 (1964).
[8] K. Heyns and D. Müller, *Tetrahedron* **21**, 3151 (1965).
[9] D. V. Bowser, R. G. Teece and S. M. Somani, *Biomed. Mass Spectrom.* **5**, 627 (1978).
[10] D. C. DeJongh, T. Radford, J. D. Hribar, S. Hanessinan, M. Bieber, G. Dawson and C. C. Seeley, *J. Am. Chem. Soc.* **91**, 1728 (1969).
[11] P. L. Coduti and C. A. Buch, *Anal. Biochem.* **78**, 21 (1977).
[12] N. K. Kochetkov and O. S. Chizhov, *Adv. Carb. Chem.* **21**, 39 (1966).
[13] S. J. Perkins, L. N. Johnson, D. C. Philips and R. A. Dwek, *Carbohydr. Res.* **59**, 19 (1977).
[14] A. S. Shashkov, A. Ju. Evstigneev and V. A. Derevitskaya, *Ibid.* **72**, 215 (1979).
[15] P. Brajeswar and W. Korytnyk, *Ibid.* **67**, 457 (1978).
[16] R. V. Lemieux, R. D. Bundle and D. A. Baker, *J. Am. Chem. Soc.* **97**, 4076 (1975).
[17] F. Conway, R. D. Guthrie, S. D. Gero, G. Lukacs, A. M. Sepulche, E. W. Hagaman and E. Wenkert, *Tetrahedron Letters* 4879 (1972).
[18] P. B. Oelrichs, P. J. Vallely, J. K. Macleod, J. Cable, D. E. Kiely and R. E. Summons, *Lloydia* **40**, 209 (1977).
[19] P. F. Lloyd and M. Stacey, *Tetrahedron* **9**, 116 (1960).
[20] M. E. Evans, *Carbohydr. Res.* **21**, 473 (1972).
[21] K. Yamamoto and T. Hayashi, *Bull. Chem. Soc. Japan* **46**, 656 (1973).