42. Cardenolides of *Asclepias syriaca* L., Probable Structure of Syrioside and Syriobioside

Glycosides and Aglycones, 334th communication[1])

by **Peter Brown**[2]), **Josef von Euw**[3]), **Tadeus Reichstein**[3]), **Klaus Stöckel**[4]) and **Thomas Robert Watson**[5])

Dedicated to Dr. *Miriam Rothschild,* who first emphasized the relation of insects to their poisonous food plants, and also initiated this study; to her 70th birthday.

(19.XII.78)

Zusammenfassung

Aus den oberirdischen Teilen der Seidenpflanze, *Asclepias syriaca* L. *(Asclepiadaceae)* isolierten *Masler et al.* [2] [3] fünf krist. Cardenolide, u. a. Syriobiosid und Syriosid, denen sie die Formeln **5** und **6** zuschrieben. *A. syriaca* ist eine der Futterpflanzen, auf denen die Larven von Schmetterlingen leben, welche die Cardenolide der Nahrung zu speichern vermögen und dadurch von der Vertilgung durch insektenfressende Tiere (bes. Vögel) teilweise geschützt sind. Die Inhaltsstoffe der Pflanze variieren stark. Bei dem uns zur Verfügung stehenden Material enthielten Blätter und Stengel nur Spuren von Cardenoliden, relativ viel enthielten die Wurzeln. Aus solchen konnte krist. Syriosid sowie eine Spur krist. Syriobiosid direkt durch Chromatographie gewonnen werden, die Hauptmenge des letzteren wurde erst nach fermentativem Abbau mit β-Glucosidasen erhalten. Chemische und physikalische Methoden zeigten, dass die vorgeschlagenen Formeln **5** und **6** unrichtig sind. Syriobiosid besitzt vermutlich Formel **7** und Syriosid Formel **10**. Letzteres liefert bei fermentativem Abbau mit β-Glucosidasen nicht Syriobiosid, wie die tschechischen Autoren glaubten, sondern einen um zwei H-Atome ärmeren Stoff, den wir Desglucosyriosid (**12**) nennen. Die Formeln sind gut begründet, aber nicht eindeutig bewiesen. Syriosid und Syriobiosid enthalten somit als Zuckerbaustein eine 4,6-Didesoxy-hexosulose (**33**), wie sie im Gomphosid (**20**) und den *Calotropis*-Cardenoliden (**22**, **24** *etc.*) vorkommt, die ebenfalls von den Larven der genannten Schmetterlinge mit der Nahrung aufgenommen werden und als Abwehrstoffe wirksam sind.

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[1]) 333. Commun. *T. Reichstein*, see Chem. Abstr. *84*, 86699 (1976).

[2]) Department of Chemistry, Arizona State University, Tempe, Arizona 85281, USA.

[3]) Institut für Organische Chemie der Universität, St. Johanns-Ring 19, CH-4056 Basel.

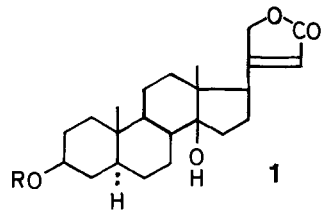
[4]) Present address: State University of New York at Buffalo, H. 513 Cooke-Hochstetter Complex, Amherst, N.Y. 14260, USA.

[5]) Pharma Department, The University of Sydney, N.S.W. 2006, Australia.

Asclepias syriaca L. (=*A. cornuti* DEC.), a plant native to N-America (see [1], p. 105–108), but introduced and widespread in the old world is known to contain cardenolides [2–8] and pregnaneglycosides [6]. We discuss here only the former. *Masler et al.* [2] [3] have isolated from the aerial parts five crystalline compounds in low yield: uzarigenin (**1**), desgluco-uzarin (**2**), syriogenin (**3**), syriobioside (putative **5**) and syrioside (putative **6**). *Mitsuhashi et al.* [6] found some xysmalogenin after acid hydrolysis of Japanese material. At the time when *Masler et al.* published their work, the structure of **1** (='odorogenin-B') was known [9]. The authors attributed structures **2**, **3**, **5** and **6** to the four new compounds. Stucture **2** for desgluco-uzarin was proved by identification of cleavage products. The tentative structure **3** was assigned to syriogenin [3b] from analytical and rotation values, spectra *etc. Brüschweiler et al.* [10] raised some doubts on the location at C(12) of one HO-group in syriogenin because two distinct peaks in the mass spectrum did not seem to be compatible with such a structure. Only later it was realized that these peaks mainly originated from slight impurities and the structure **3** was shown to be correct independently by *Casagrande et al.* [11] and *Okada & Anjyo* [12]. *Masler et al.* [3] suggested formulae **5** and **6** for syriobioside and syrioside, but these must be revised (see below).

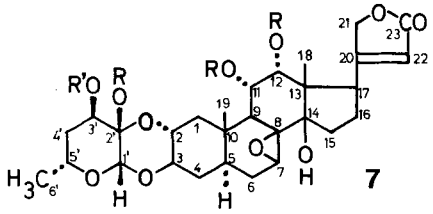
In connection with work on certain insects which are capable of storing cardenolides ingested by their larvae feeding on milkweeds (different representatives of *Asclepiadaceae* and related plant genera), we were interested in syrioside and syriobioside, because *Asclepias syriaca* is one of the main natural food plants of the Monarch butterfly (*Danaus plexippus* L.) and other poisonous insects which behave in this manner (see [7] [8] [13] [14] and further lit. in [8], p. 122). It is known that the amount of cardenolides in this plant is very variable (see [8], p. 105, 122 *etc.*). For isolation work we used a crop of *A. syriaca* of garden origin cultivated for many years in Basel by the senior author *(T.R.)*. Dried leaves and stems contained only small amounts of cardenolides, but roots gave better although somewhat erratic results. From 670 g dried roots dug up in fall 1973 we could isolate 271 mg of crystalline syrioside after one single chromatography. Only traces of free syriobioside could be detected in this material. A little more was present as a D-gluco-derivative (**9**) as treatment of the highly polar amorphous material (dried mother liquors of syrioside) with β-glucosidases (snail enzyme or commercial 'cellulase') gave mixtures from which a total of 53 mg of pure crystalline syriobioside could be obtained after repeated chromatography. From 820 g dried roots harvested in fall 1974 only 35 mg of crystalline syrioside were isolated, and tedious partition chromatography was necessary to separate it. No pure syriobioside was secured in this experiment. This second batch obviously contained relatively more *'Kedde'* – negative material (perhaps polyhydroxy-pregnane glycosides, see *Mitsuhashi et al.* [6a] and *Papay et al.* [6b]) – which impedes isolation of cardenolides. The identity of our crystals was established by comparison with authentic samples of syriobioside, syrioside and syrioside-acetate kindly provided by Dr. *S. Bauer*. Melting points and rotation values[6]) of our preparations were in good agreement with values given by the Czech

[6]) *Masler et al.* [3a] give $[\alpha]_D^{22} = +11.5°$ in pyridine for syriobioside. We found $[\alpha]_D^{25} = +26.4° \pm 8°$ in this solvent after 5 min. and 0° after 30 and 45 min. The compound is obviously rapidly decomposed or rearranged in pyridine. All other compounds gave rotation values in good agreement with those reported by the Czech authors.



1 (R = H) Uzarigenin (= Odorigenin B) [9]. M.p. 243–250°, $[\alpha]_D = +14.0$ (eth.) [2] $C_{23}H_{34}O_4$ (374)

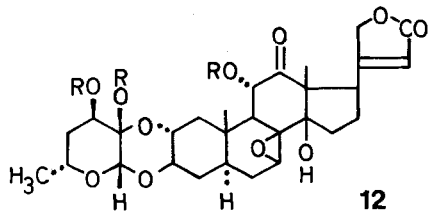
2 (R = β-D-glucosyl-) Desgluco-uzarin. M.p. 260–272°, $[\alpha]_D = -44.1$ (py.) $C_{29}H_{44}O_9$ (536) [2] [3]. Tetra-*O*-acetyl derivative m.p. 174–176°, $[\alpha]_D = -8.6$ (chlf.) $C_{37}H_{52}O_{13}$



7 (R = R′ = H)Syriobioside (TR-1525). M.p. 221–223°, $C_{29}H_{40}O_{11}$ (564) with hypothetical genin $C_{23}H_{32}O_8$ (436)

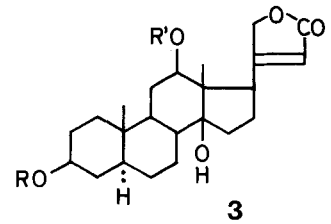
8 (R = R′ = Ac)Tetra-*O*-acetyl-syriobioside (TR-1561). M.p. 338–340°, $[\alpha]_D = +33.2$ (chlf.) $C_{37}H_{48}O_{15}$ (732) with hypothetical genin $C_{27}H_{36}O_{10}$ (520)

9 (R = H, R′ = glucosyl)Dihydrosyrioside hypothetical



12 (R = H)Desglucosyrioside (TR-1554). M.p. 204–206°, $[\alpha]_D = +26$ (chlf.) $C_{29}H_{38}O_{11}$ (562) with hypothetical genin $C_{23}H_{30}O_8$ (434)

13 (R = Ac) Tri-*O*-acetyl-desglucosyrioside (TR-1555). M.p. 302–303°, $[\alpha]_D = +3.1$ (chlf.) $C_{35}H_{44}O_{14}$ (688) with hypothetical genin $C_{25}H_{32}O_9$ (476)

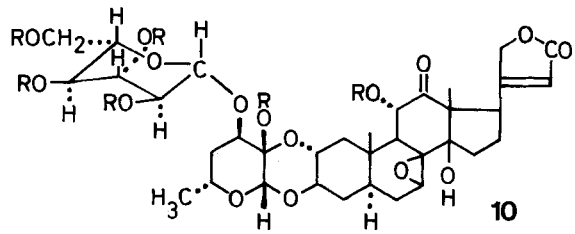


3 (R = R′ = H) Syriogenin. M.p. 278–288°, $[\alpha]_D = +9$ (py.) $C_{23}H_{34}O_5$ (390) [3a]

4 (R = R′ = Ac) Di-*O*-acetylsyriogenin. M.p. 173–174°, $[\alpha]_D = +26.2$ (chlf.) [3] $C_{27}H_{28}O_7$ (474) [3b]

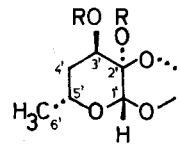
5 (R = β-D-glucosyl-L-rhamnosyl-, R′ = H) = putative formula of syriobioside [3a]. M.p. 220–222°, $[\alpha]_D = +11.5$ (py.) assumed to be $C_{35}H_{54}O_{11}$ (698.8)

6 (R = β-D-glucosyl-β-D-glucosyl-L-rhamnosyl-, R′ = H) = putative formula of syrioside [3a]. M.p. 234–237°, $[\alpha]_D = -13.6$ (py.) assumed to be $C_{41}H_{64}O_{19}$ (896.9). Acetate: M.p. 185–189°, $[\alpha]_D = -2.5$ (chlf.) assumed to be $C_{61}H_{84}O_{29}$ (1281.3) [3b]



10 (R = H)Syrioside (TR-1524). M.p. 230–231°, $[\alpha]_D = -12.8$ (py.) $C_{35}H_{48}O_{16}$ (724) with hypothetical genin $C_{23}H_{30}O_8$ (434)

11 (R = Ac)Hexa-*O*-acetyl-syrioside (TR-1527). M.p. 192–193°, $[\alpha]_D = -1.2$ (chlf.) $C_{47}H_{60}O_{22}$ (976) with hypothetical genin $C_{25}H_{32}O_9$ (476)



14 Other possibility with reversed configuration at C(2′) of sugar moiety corresponding to **28**

authors [3a, b], mixed m.p. gave no depression and Rf-values in thin layer chromatography (TLC.) and paper-chromatography (PC.) in different systems were identical.

The material allowed us to show that the tentative structures **5** and **6** suggested by *Masler et al.* [3a, b] for syriobioside and syrioside cannot be correct. Chemical degradation, combined with physical methods, particularly NMR. and mass spectra, allowed us to suggest formulae **7** and **10** as most probable structures for these compounds. X-ray work may be necessary for a final proof but we are confident that our formulae **7–13** in principle are correct, although some details (particularly chirality at C(2′) of the sugar moiety) need further study, and even a rigid proof that the sterol nucleus is present is still missing.

Masler et al. [3a, b] claim to have identified syriogenin (**3**) rhamnose and glucose by PC. after mild hydrolysis (method of *Mannich & Siewert* [15]) of syriobioside, and also after vigorous hydrolysis of syrioside with 5% sulfuric acid in aqueous ethanol. They also claim to have obtained crystalline syriobioside (m.p. 219–222°) after enzymatic cleavage of syrioside with appropriate β-glucosidases (snail enzyme or preparation from *Adonis vernalis* [16]).

In our hands no rhamnose nor any other 'normal' sugar could be traced in PC. (methods see [17]) after vigorous hydrolysis of syriobioside (**7**) with *Kiliani*-mixture on a micro scale [18]. Syrioside (**10**) under these conditions gave D-glucose as sole 'normal' sugar, and this could be isolated as crystalline penta-*O*-acetyl-D-glucose by acetolysis of hexa-*O*-acetyl-syrioside (**11**) on a preparative scale. Syrioside (**10**) after treatment with β-glucosidases (snail enzyme or commercial 'cellulase'[7]) gave D-glucose and a compound which we call desgluco-syrioside (**12**). It has similar chromatographic properties and similar m.p. as syriobioside (**7**) but is slightly less polar and contains 2 H-atoms less. Small amounts of free **12** are also present in the roots. Exhaustive acetylation with acetic anhydride in pyridine at 35° for 6 days gives a tri-*O*-acetyl-derivative (**13**) while syriobioside (**7**) under these conditions yields a tetra-*O*-acetyl-derivative (**8**) with a distinctly higher rotation. No 'normal' sugar could be detected after vigorous hydrolysis of desgluco-syrioside (**12**).

On the other hand syriobioside (**7**) and desgluco-syrioside (**12**) gave a very strong positive 'osazone reaction for methyl-reductinic acid' (see *Hesse et al.* [19], particularly p. 74 and 86). This can be performed on micro scale and is typical for the hexosulose moiety (**15**) in the *Calotropis* glycosides, *e.g.* calactin (**22**), calotropin (**24**), proceroside [10], as well as in gomphoside (**20**) and afroside from *Gomphocarpus fruticosus* [20–22], see *Brüschweiler et al.* (see [23], table 1, p. 2777). All these compounds are decomposed on heating into 'Herzgift-methylreductinsäure' (**18**) [24–28] and the genin (**19**), a reaction for which *Crout et al.* [29] [30] suggested a mechanism corresponding to **15–19**. This thermal reaction can easily be observed by prominent peaks at *m/e* 128 and 113 (= 128–15) in electron impact (EI) mass spectra (provided ion detection is performed quickly after introducing the probe [31] [10] [23]) while peaks of the molecular ion or the genin (**18**) are usually weak or absent in this procedure. As in other cardenolide glycosides [32] field ionisation (FI) gave more informative results. Other 'soft' methods (see review [33]) may be as good. Although

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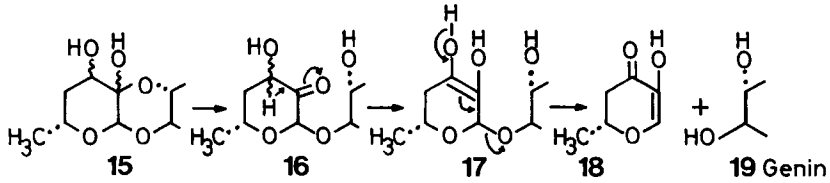
[7]) We thank the *Ferment AG* Basel for a gift of this very active material prepared from *Aspergillus spec.*

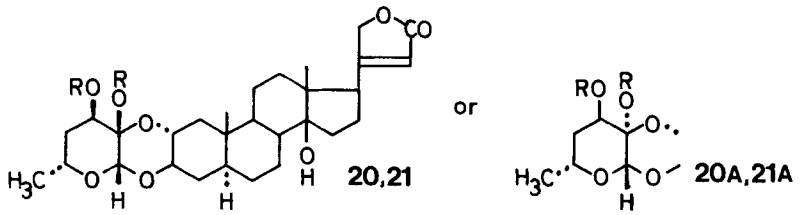


(M.p. 83,
$[\alpha]_D = +134$–146 (meth.)
$C_6H_8O_3$ (128) [24–28])

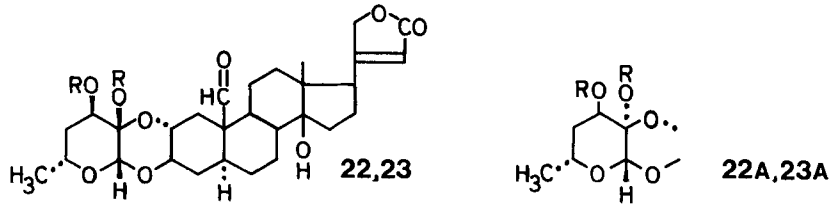
molecular ions are still weak or absent in FI-mass spectra of this kind of compounds; they all showed strong peaks for **18** and the genin (**19**) (or genin-18). Molecular ions can often be observed in the *O*-acetyl-derivatives (see below).

Both syriobioside (**7**) and desgluco-syrioside (**12**) behaved exactly in this manner (see *Fig. 4* and *5*). We accept this as strong evidence that they may contain a similar sugar moiety. This was fully substantiated by the NMR. spectra (see below). As no other sugar could be detected in **7** and **12** we further conclude that both must contain highly oxygenated genins to explain their high oxygen content as found in combustion analysis [3]. This is fully confirmed from the mentioned mass spectra *(Fig. 4* and *5)* and results of acetylation (see below). The name syriobioside for compound **7** is therefore misleading as it contains only one sugar, but nevertheless we continue to use it for historical reasons.

In the following discussion we give our reasons for assigning structures **7**, **12** and **10** to syriobioside, desgluco-syrioside and syrioside.

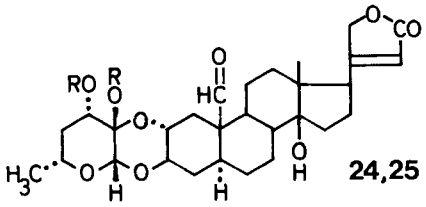


20 or **20A** (R = H)Gomphoside, m.p. 232–243°, $[\alpha]_D^{8} = +15$ (CH_3OH) $C_{29}H_{42}O_8$ (518) [20b,c] [21][8])
21 or **21A** (R = Ac)Di-*O*-acetyl-gomphoside, m.p. 252–255°, $[\alpha]_D^{20} = +32$ (chlf.) $C_{33}H_{46}O_{10}$ (602) [20–22][9])

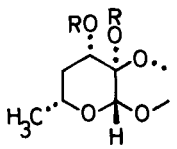


22 or **22A** (R = H)Calactin, m.p. 262–267°, $[\alpha]_D = +57.3$ (CH_3OH) $C_{29}H_{40}O_9$ (532) [23]
23 or **23A** (R = Ac)Di-*O*-acetyl-calactin, m.p. 252–254°, $[\alpha]_D = +42.3 \rightarrow 38.1$ (chlf.)[10]) $C_{33}H_{44}O_{11}$ (614)

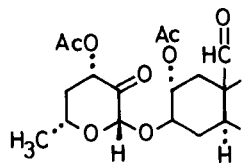
[8]) A slightly wrong formula $C_{29}H_{44}O_8$ (520) based on combustion analysis is given in [20c].
[9]) Empirical formula $C_{33}H_{48}O_{10}$ (604) derived from combustion analysis is given in [20c].
[10]) Whether the 'mutarotation' (own measurement) is real (possible addition of methanol?) could not be checked.



24,25

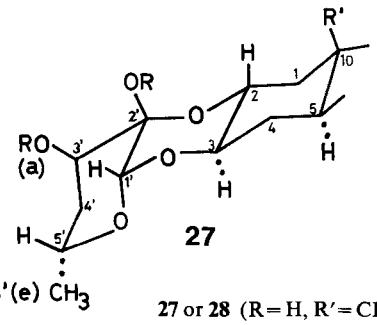


24A,25A



26 [36]

24 or **24A** (R = H)Calotropin, m.p. 221°, $[\alpha]_D = +65.3$ (CH_3OH) $C_{29}H_4O_9$ (532) [23]
25, **25A** or **26** (R = Ac)Di-*O*-acetyl-calotropin, amorph., $[\alpha]_D = 0°$ (chlf.) $C_{33}H_{44}O_{11}$ (614)



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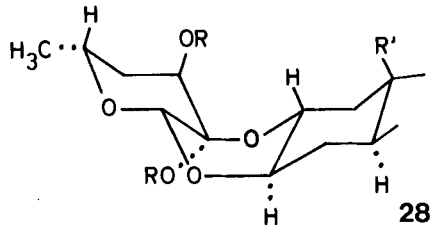
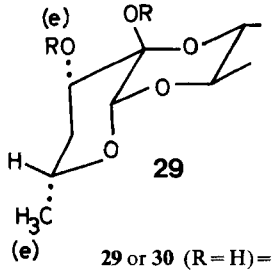


28

27 or **28** (R = H, R′ = CH_3) = Gomphoside (**20** or **20A**)
(R = Ac, R′ = CH_3) = Di-*O*-acetyl-gomphoside (**21** or **21A**)
(R = H, R′ = CHO) = Calactin (**22** or **22A**)
(R = Ac, R′ = CHO) = Di-*O*-acetyl-calactin (**23** or **23A**)



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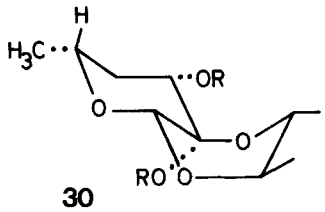

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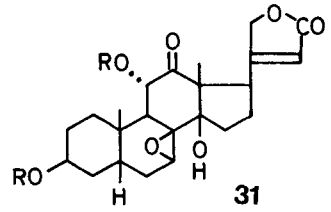
29 or **30** (R = H) = Calotropin (**24** or **24A**)
(R = Ac) = **25** or **25A**



31

31 (R = H) = Sarverogenin, m.p. 136/227°, $[\alpha]_D = +47.2$ (CH_3OH) $C_{23}H_{30}O_7$ (418) [37]
32 (R = Ac) = Di-*O*-acetyl-sarverogenin, m.p. 268°, $[\alpha]_D = +21.6$ (CH_3OH) $C_{27}H_{34}O_9$ (502) [37]

Biological activity of **7** and **10** has obviously never been checked. We could get at least some values for syrioside (**10**). In isolated spontaneously beating guinea pig atria [34] it induced a concentration dependent digitalis-like increase in the amplitude of contraction but was less potent than digitoxin[11]), while in the ATP-ase test [35] it was as active as digitoxin[12]). Digitalis-like activity has so far only been found in steroids containing a butenolide side chain, and a 14β-hydroxy group. The pres-

[11]) We thank P.D. Dr. *G. Scholtysik* from the Biological and Medical Research Division, *Sandoz AG* Basle, for performing the experiments and allowing us to publish them (in litt. 7.1.1976, 20.12.1977).

[12]) We thank Dr. *H.J. Portius* and Prof. Dr. *K.R.H. Repke*, Forschungszentrum für Molekularbiologie und Medizin, Akad. der Wiss. Berlin-Buch, DDR, very much for investigating our compound and sending us the results (in litt. 4.6.1976).

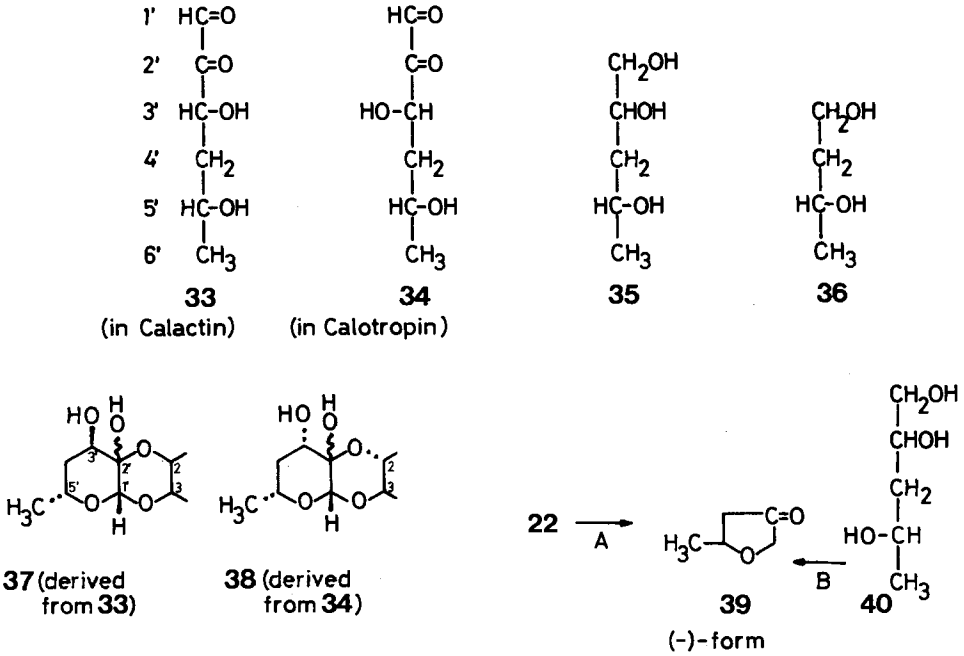
ence of the butenolide ring is also confirmed by the UV. [3] [6] and IR. spectra (see *Fig. 1–3*). All of the compounds showed IR. bands typical for the butenolide ring, but some other regions, particularly those around 2800–3000 cm^{-1}, were poorly resolved, probably due to presence of water of crystallisation. This made it difficult also to check the empirical formulae by combustion analysis. To prevent thermal decomposition all samples were dried at 20°/0.01 Torr over P_2O_5 for 24 h. Except compound **13** which gave a correct result for $C_{35}H_{44}O_{14}$, all other compounds gave values corresponding to one molecule of H_2O in excess to those calculated. We assume that this is not covalently bound but rather firmly bound water of crystallisation, as otherwise it would be difficult to explain the results of mass spectra. Free water was also visible in the ^{1}H-NMR. spectra.

Acetylations. For structure determinations the *O*-acetyl derivatives have been used extensively. Acetylation of the sugar moiety proceeds stepwise, the secondary HO-group at C(3′) reacts quickly, the angular one at C(2′) rather slowly as observed by *Brüschweiler et al.* (see [23], p. 2282) for compounds of this type and by *Singh & Rastogi* [36] for calotropin (**24**) in particular. In some cases the separation of fully and partially acetylated compounds is difficult. In order to get pure material in high yield we sealed the samples with excess of acetic anhydride and abs. pyridine *in vacuo* and kept them at 35° for 8 days (see [23], p. 2282). The HO-group at C(14) is not attacked under these conditions nor is the aldehyde group in **22** and **24** which would partially be destroyed by autoxidation if oxygen were not excluded.

Model compounds. For interpretation of the results of NMR. and mass spectra we used gomphoside (**20**), calactin (**22**), calotropin (**24**), sarverogenin (**31**) and their *O*-acetyl derivatives **21**, **23**, **25** or **26** and **32** as models with essentially known structures. In formulae **20–25** some details given in the original literature (reviewed in [23]) are altered to fit new results. Structures of the genins of **20**, **22** and **24** (gomphogenin and calotropagenin) as well as of sarverogenin (**31**) are established (reviewed in [23]). Structure of the sugar is not completely proved, and our present formulation in **20–24** is based on the following facts:

Calactin (**22**) and calotropin (**24**) produce identical products, compound **18** and calotropagenin [24–26], by pyrolysis. They can therefore differ only in the configuration at C(1′), C(2′) or C(3′) which loose their chirality when transformed into **18**. As shown by *Crout et al.* [29] [30] they are derived from 4,6-dideoxy-hexosuloses. For these sugars only the two formulae **33** and **34** (corresponding to the D-series written in *Fischer* projection) are possible, the configuration at C(5′) being established in two ways. *Crout et al.* [38] obtained (4*R*)-pentane-1,2,4-triol (**35**), $[\alpha]_D = -12°$ by degradation of calactin (**22**) and *Coombe & Watson* [21] isolated (−)-D-butane-1,3-diol (**36**) starting with gomphoside (**20**). An earlier report by *Curtis et al.* [38a] seems at first sight to be in contradiction with these results. These authors [38a] obtained the same optically active (−)-tetrahydro-3-oxofuran (**39**) in several steps both from calactin (**22**) and from (+)-(4*S*)-pentane-triol (**40**). We assume that in one of the two series of steps (A or B) inversion at the remaining center of chirality must have taken place.

The two possible sugars (**33** or **34**) in the original glycosides are bound to the 2*α*,3*β*-dihydroxy-steroid through glycoside and half acetal bonding producing the dioxane derivatives **37** or **38**. The NMR. signals (see *Table 4*) of the sugar moiety in



gomphoside (**20**) and calactin (**22**), as well as in their di-*O*-acetyl derivatives **21** and **23**, are virtually at the same chemical shift which we accept as evidence that those two compounds have identical structures in their sugar moiety and differ only at C(10) of the genin where gomphoside (**20**) carries a methyl group, and calactin (**22**) an aldehyde group. From the NMR. spectrum it is also evident that the HO-group at C(3′) is axial in these two compounds while it is equatorial in calotropin (**24**) (see below). So far it is impossible to assign reliable configurations at C(1′) and C(2′) with the available spectra, but according to *Klyne*'s rule [39] all natural cardenolide glycoside have at C(1′) the same absolute configuration, *i.e.* *β*-D or *α*-L. As we are in the D-series, we prefer to suggest *β*-D which, in the steroid type of writing, gives **37** and **38** with *β*-H orientation at C(1′). Assuming a chair conformation of the sugar moiety this brings also the methyl group at C(6′) in the preferred equatorial position (**27**–**28** or **29**–**30** resp.).

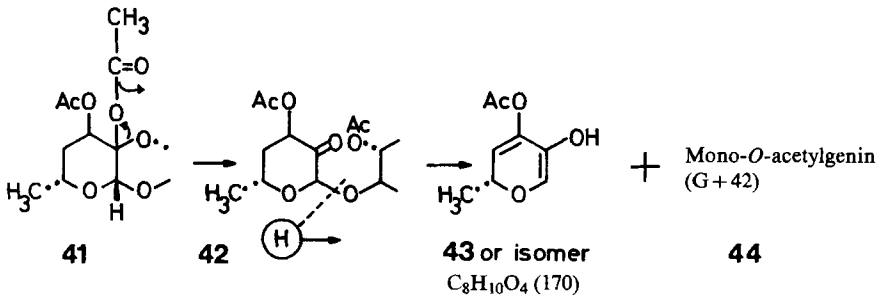
Still less evidence is available for chirality at C(2′). *Coombe & Watson* (see [21], p. 95) state that the diol system in gomphoside (**20**) does not form an acetonide, suggesting a *trans*-position, so formulated by *Brüschweiler et al.* [23]. We now prefer not to attribute too much weight to the nonreactivity with acetone, as it could be due to steric hindrance, and so leave the decision open. Gomphoside would then be either **20** or **20A**. For **20** the all chair conformation (**27**) would probably be the preferred arrangement while for **20A** a boat conformation of the dioxane ring corresponding to **28** may also be favorable. Some ^{13}C-NMR. data of the acetate seem to indicate that **27** (= **21**) is more likely to be correct than **28** (= **22**).

As mentioned above NMR. spectra *(Table 4)* show that the acetoxy group at C(3′) in di-*O*-acetyl-gomphoside (**21**) and in di-*O*-acetyl-calactin (**23**) is axial, while

in di-*O*-acetyl-calotropin (**25** or **26**) it is equatorial. *Singh & Rastogi* [36] suggested that the dioxane ring of calotropin (**24**) is opened during acetylation and di-*O*-acetyl-calotropin has structure **26**. Their argument is based on the pronounced downfield shift of the signal for the H–C(1′) of calotropin (**24**) in the NMR. spectrum after acetylation. This explanation may be correct but the shift could also be reconciled with structure **25**. We had not sufficient material of di-*O*-acetyl-calotropin to check this structure by a ^{13}C-NMR. spectrum, and leave the decision open. All of the other compounds **20**, **22** as well as **7**, **10** and **12** did not show this shift *(Table 4)*, and there is no reason to assume they should rearrange in the suggested way during acetylation. For di-*O*-acetyl-gomphoside (**21**) and di-*O*-acetyl-calactin (**23**) the ^{13}C-NMR. spectra (see *Table 3*) are in agreement with the given structures and show no signals which could be assigned to a keto group at C(3′). We are therefore confident that the same is true for **8** and **13** as the signals for their sugar moieties in the ^{1}H-NMR. spectra (see *Table 4*) are virtually at the same positions.

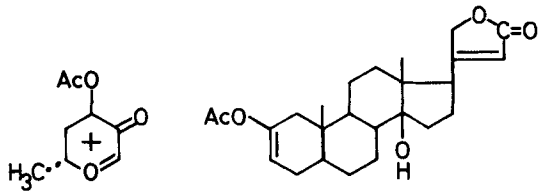
Mass spectra of free glycosides. In the FI-spectrum of syriobioside (**7**) *(Fig. 4)* no molecular peak is visible, but a weak one for $M-18$ is present. Besides a prominent peak at *m/e* 128 (**18**) there is a very strong one at *m/e* 436 ($=C_{23}H_{32}O_8$, *Table 2*) indicative of a genin. Desglucosyrioside (**12**) *(Fig. 5)* gave a small peak for the molecular ion, and a strong one at *m/e* 128 (**18**) ($=C_6H_8O_3$, *Table 2*), 416 ($C_{23}H_{23}O_7$, *Table 2*), and a medium one at *m/e* 434 indicative of the genin. In the FI-spectrum of syrioside (**10**) *(Fig. 6)* no molecular ion is visible but again prominent peaks at *m/e* 128 (**18**) and 416 ($C_{23}H_{28}O_7$, *Table 2*) corresponding to genin-18 are present.

Mass spectra of acetyl derivatives. In the acetylated compounds (**8**, **11**, **13**, **21**, **23** and **25**) a thermal reaction corresponding to scheme **15** → **18** is only possible after elimination of ketene, or the glucosyl residue in **11**. This may indeed take place in the FI-spectra to a small extent as fairly intense peaks at *m/e* 170 (**43** or isomer), and minor ones for the genin (G) were observed in all cases. Another reaction only observed in the FI-spectra gives rise to peaks at *m/e* 170, and genin +42; this we tentatively formulate as an acetyl shift **41** → **42** producing an intermediate **42** corresponding to *Singh & Rastogi*'s formula for di-*O*-acetyl-calotropin (**26**) leading to fragments **43** and **44**. This may be a thermal process as production of the ions **43** and **44** can be minimized by lowering the ion source temperature, but it could be due to processes occurring in the FI-emitter as it was not observed in EI and H_2-CI. (chemical ionisation using hydrogen as ionising gas) mass spectra (see *Fig. 8* and *11*). In the H_2-CI. spectrum of di-*O*-acetyl-gomphoside (**21**) as a model *(Fig. 8)* a distinct



41 **42** **43** or isomer $C_8H_{10}O_4$ (170) **44**

peak of $M+H^+$ is visible, the other most prominent peaks are attributable to loss of H_2O, and one or two mol CH_3COOH. Peaks for $G+H^+$ (391) or $G+42+H^+$ (433) are absent, but a distinct peak at *m/e* 171 ($43+H^+$) perhaps best formulated as **45** is again visible. From the metastable ions obtained in defocussed spectra (see *Table 1*) it can be deduced that of the four most probable processes for forming the ion *m/e* 171 (as depicted in *Table 1*) only the following two can be observed: 585 ($M+H^+-18$) $\xrightarrow{-414}$ 171 and 525 (585–60) $\xrightarrow{-354}$ 171. No peak (of intensity >0.3%) for the loss of fragment 414 corresponding to **46** is apparent but a distinct peak at *m/e* 355 corresponding to a di-anhydro-genin (390–36, or **46** –60) perhaps $47+H^+$ is observed. If this interpretation is correct, the formation of the lost frag-

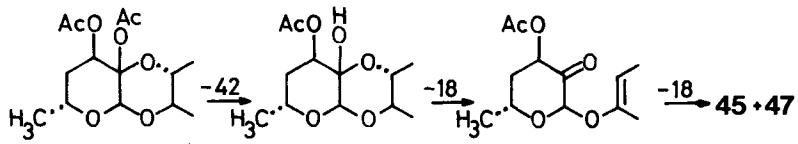


45 ($S-18+42+H^+$) $C_8H_{11}O_4$ (171)

46 or isomer ($G-18+42$) $C_{25}H_{34}O_5$ (414) (for di-*O*-acetyl-gomphoside)

47 or isomer $G-36$ $C_{23}H_{30}O_3$ (354) (for di-*O*-acetyl-gomphoside)

ment *m/e* 414 (perhaps **46**) even if not directly visible in the spectrum is indicative of some acetyl-migration after, or concomitant with, loss of water in **21**. But the directly visible fragments in the H_2-CI. spectrum are **45** and $47+H^+$ (355) which are both probably formed without acetyl-migration perhaps *via* the intermediates **49** and **50**, or in a concerted process **46** $\xrightarrow{-60}$ **47**.



48 **49** **50**

With these results from our model compounds we can interpret the structures of syriobioside (**7**), desgluco-syrioside (**12**) and syrioside (**10**) with some confidence.

Syriobioside (**7**). The empirical formula $C_{29}H_{40}O_{11}$ (564) is evident from addition of the fragments **18** ($C_6H_8O_3$) and genin ($C_{23}H_{32}O_8$) seen in its FI-mass spectrum (*Fig. 4* and *Table 2*). Probable structure of the sugar moiety is discussed above. The genin has two H-atoms less than $C_{23}H_{34}O_8$ calculated for a hexahydroxy-cardenolide. If our assumption is correct that it really is a normal cardenolide, then a double bond, a carbonyl group or an epoxy ring must be present. Although evidence for the absence of a double bond (negative $C(NO_2)_4$ reaction in the *O*-acetyl-derivative) and a carbonyl group (UV. and IR. spectra) are meagre due to insufficient amount of material (no ^{13}C-NMR. spectrum possible), we postulate the presence of a 7β, 8β-oxirane ring on the basis of the ^{1}H-NMR. spectrum of the *O*-acetyl-derivative. Acetylation of syriobioside (**7**) gave a tetra-*O*-acetyl-derivative (**8**) which in the FI-

mass spectrum *(Fig. 9)* showed a small but distinct molecular ion, and asside from other peaks distinct peaks for the genin (520), and genin +42 (562), probably formed through acetyl-migration corresponding to **41** and to **42** (170). The ^{1}H-NMR. spectrum *(Fig. 14)* shows four signals for acetyl groups of which two must be in the sugar moiety. The other two are most compatible with positions 11 and 12 in the sterol nucleus. As an 11β-hydroxy group is not acetylated under our conditions, it must be in the 11α orientation. From the coupling constants it is also evident that these two groups are *cis, i.e.* 11α, 12α, a rare arrangement in natural steroids. An important signal in the spectrum *(Fig. 14)* is the doublet at $\delta = 3.38$ ppm ($J = 6$) which we take as strong evidence for the presence of the 7β, 8β-oxirane ring, as it is in excellent agreement, with the similar signal in the spectrum of di-*O*-acetyl-sarverogenin (**32**) [37] (see *Table 4*).

Desglucosyrioside (**12**). This compound gave a peak corresponding to the molecular ion ($C_{29}H_{38}O_5 = 562$) in the FI-mass spectrum *(Fig. 5)* and strong peaks for the fragments **18** and **19** (genin $C_{23}H_{30}O_8 = 434$). The latter therefore contains two H-atoms less than syriobioside (7). Acetylation gave a tri-*O*-acetyl-derivative (**13**) showing only three signals for acetyl groups in the ^{1}H-NMR. spectrum *(Fig. 16* and *Table 4)* and in the FI-mass spectrum *(Fig. 10)* peaks for a molecular ion (688), genin (476), mono-*O*-acetyl-genin (518) and **41** (170). In the H_2-CI. mass spectrum *(Fig. 11)* corresponding peaks were only visible for $M+1$ (689, very weak) and *m/e* 171 (**43**) while no peaks for genin + 1 (477) or mono-*O*-acetyl-genin + 1 (519) were detected. The presence of a keto group (assigned to 12-position) is clearly visible in the IR. and the ^{13}C-NMR. spectrum *(Table 3)* and the 11β-(axial) proton as a doublet ($\delta = 5.66$; $J = 11.5$) in the ^{1}H-NMR spectrum *(Fig. 16* and *Table 4)*. These assignments are in good agreement with the spectrum of di-*O*-acetyl-sarverogenin (**32**) [37] (see *Table 4*), and small differences are probably mainly due to the different configuration at C(5). The ^{13}C-NMR. spectrum *(Table 3)* is quite compatible with the all chair conformation (**27**) of structure **13**, and probably better than with structure **14** (with the dioxane ring as a boat (**28**)) without excluding the latter.

Syrioside (**10**). This compound contains one mol D-glucose bound glycosidically to **12**. From its relative stability to hydrolysis (excludes a furanoside), rotation differences, and the ^{1}H-NMR. spectrum of its hexa-*O*-acetyl-derivative *(Table 4)*, we conclude that it is bound as a β-D-glucopyranoside. The glucose molecule is not attached to at 11α because in the ^{1}H-NMR. spectrum (see *Table 4*) of the hexa-*O*-acetyl-derivative (**11**), the signal of the 11β-H is virtually at the same position as in **13**. We have therefore suggested tentatively that the glucose is attached in 3′ position as the most likely place, but no final proof is available. The FI-mass spectrum of free syrioside (**10**) *(Fig. 6)* shows no peak of a molecular ion (as expected) and even no peak for the genin (434) but strong peaks for G-18 (416) *etc.*, and **18** (128) and also a small one for the glucosyl cation (163). In the FI-mass spectrum of the hexa-*O*-acetyl-derivative (**11**) *(Fig. 12)* again no molecular ion (976) is visible, only small peaks for $M-60$ and $M-120$, but distinct peaks for other fragments including mono-*O*-acetyl-genin (518), genin (476) *etc.*, anhydro-tetra-*O*-acetyl-glucose (S2 = 330) and **41** (170) are present. ^{1}H-NMR. spectrum (see *Table 4*) of hexa-*O*-acetyl-syrioside (**11**) is in good agreement with the suggested structure and confirms the presence of six acetyl groups.

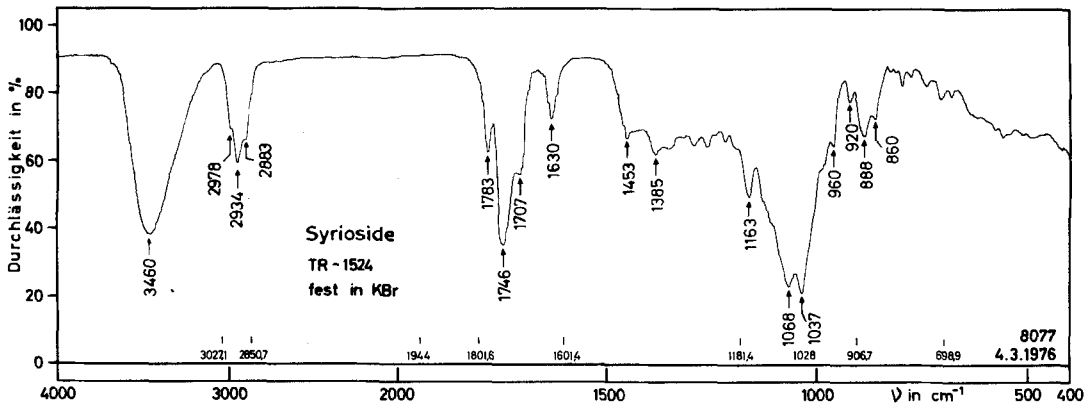
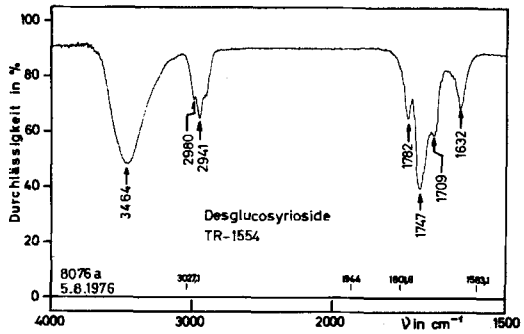


Fig. 1. *IR. spectrum of syrioside* (**10** = TR-1524), m.p. 230–231°, 0.72 mg crystals pressed in *ca.* 300 mg KBr[13]). Resolution is bad probably owing to water of crystallisation. The peaks at *ca.* 1783, 1746 and 1630 cm^{-1} correspond to the butenolide-ring and 1707 to the keto-group at C(12).



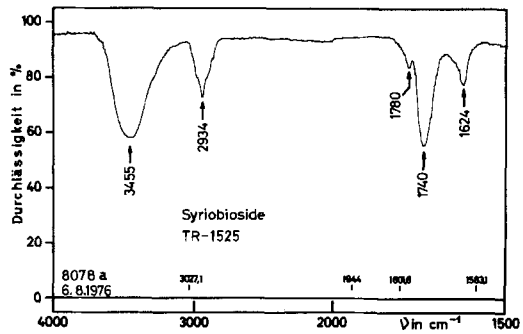


Fig. 2–3. *Partial IR. spectra of desglucosyrioside* (**12** = TR-1554) *(Fig. 2)* [m.p. 204–206°, 0.65 mg crystals pressed in *ca.* 250 mg KBr[13]) showing bands at *ca.* 1782, 1747 and 1632 cm^{-1}, corresponding to the butenolide-ring, and 1709 to the keto-group at C(12)] *and syriobioside* (**7** = TR-1525) *(Fig. 3)* [m.p. 221–223°, 0.45 mg crystals pressed in *ca.* 200 mg KBr[13]) showing only the butenolide-bands at *ca.* 1780, 1740 and 1624 cm^{-1}. No band of a keto-group at *ca.* 1708 cm^{-1} is visible]

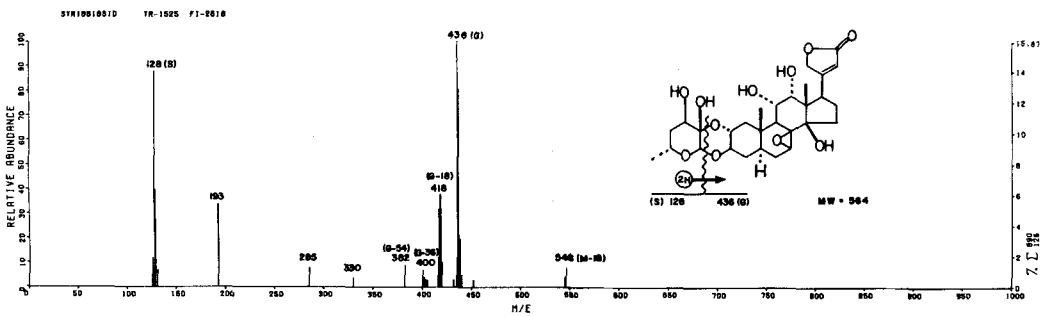


Fig. 4. *FI-mass spectrum*[14]) *of syriobioside* (**7** = TR-1525), m.p. 221–223°, $C_{29}H_{40}O_{11}$ (564), probe temp. 250°. Assignments: M^+ not observed; 546 ($M-18$); 436 (G); 418 (G – 18); 400 (418–18); 382 (400–18); 128 (**18**).

[13]) Recorded by *K. Aegerter* on a *Perkin-Elmer* IR. Spectrophotometer model 125.

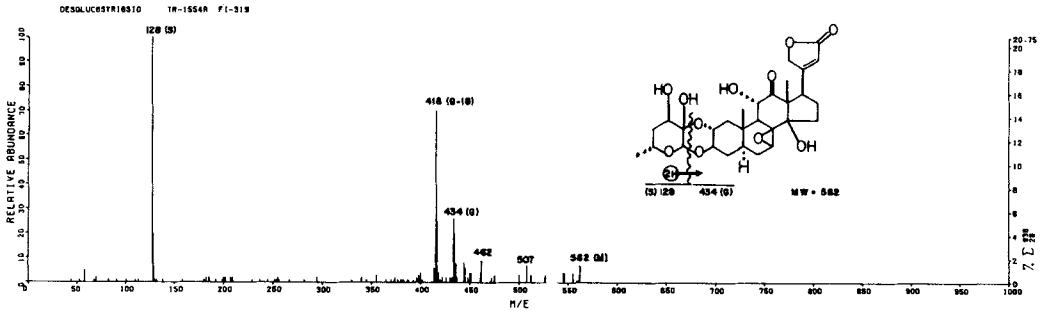


Fig. 5. *FI-mass spectrum*[14]) *of desglucosyrioside* (**12** = TR-1554A), m.p. 204–206°, $C_{29}H_{38}O_{11}$ (562), probe temp. 235°. Assignments: 562 (M); 434 (G); 416 (G – 18); 128 (**18**).

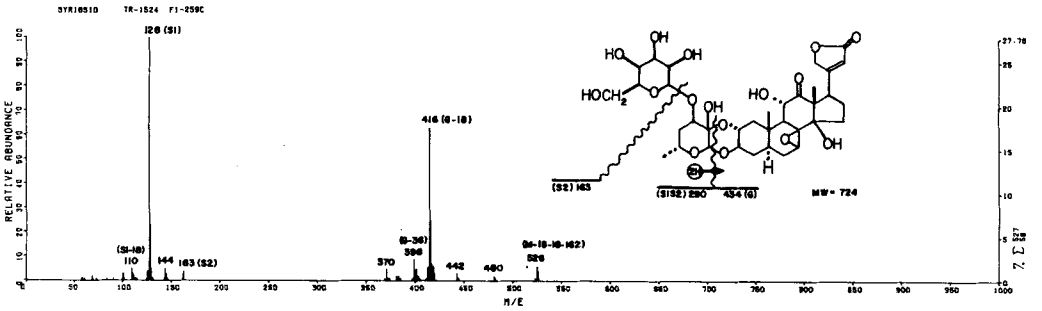


Fig. 6. *FI-mass spectrum*[14]) *of syrioside* (**10** = TR-1524), m.p. 230–231°, $C_{35}H_{48}O_{16}$ (724), probe temp. 235°. Assignments: 526 ($M-18-180$ ($C_6H_{12}O_6$ = glucose)); 416 (G – 18); 398 (416–18); 370 (398–28); 163 ($C_6H_{11}O_5$ = glycosyl cation); 128 (**18**).

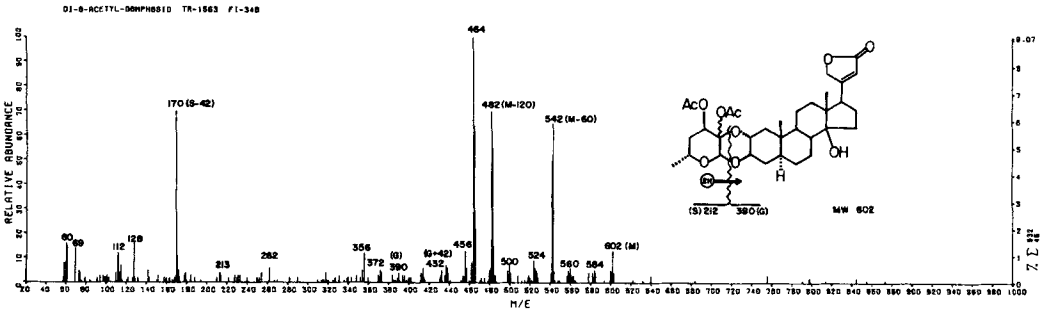


Fig. 7. *FI-mass spectrum*[14]) *of di-O-acetyl gomphoside* (**21** = TR-1563 purified by chromatography on SiO_2), m.p. 285–287°, $C_{33}H_{46}O_{10}$ (602), probe temp. 220°. Assignments: 602 (*M*); 584 (*M* – 18); 560 (*M* – 42); 542 (*M* – 60); 524 (542–18); 500 (542–42); 482 (*M* – 120); 464 (482–18); 456 (500–44); 432 (G + 42); 390 (G); 372 (G – 18); 356 (?); 170 (**43**).

[14]) Secured by Mr. *Richard B. Scott.* Details see exper. part. Composition of ions by high resolution mass spectroscopy (see *Table 1*).

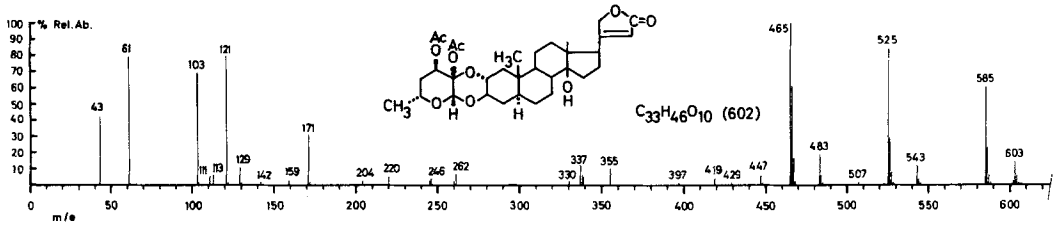
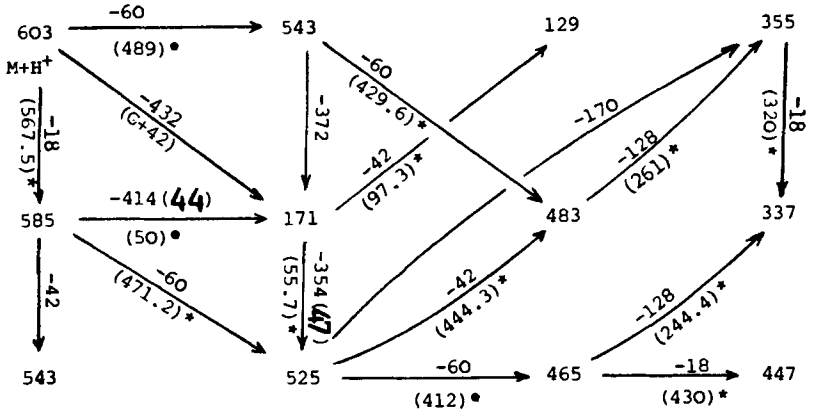


Fig. 8. *H_2-CI.-mass spectrum*[15]) *of di-O-acetyl-gomphoside* (**21**), source temp. 220°, probe temp. 180°. Assignments: 603 ($M+H^+$); 585 (603–18); 543 (603–60 and 585–42); 525 (585–60); 501 (543–42); 483 (543–60); 465 (525–60 and 483–18); 447 (465–18); 429 (447–18); 397 (525–128); 355 (483–128 and 525–170, corresp. to **47** (= G – 36) + H^+); 337 (465–128 and 355–18 and 525–170); 171 (**43**); 129 (171–42); 111 (129–18). The processes summarized in *Scheme 1* could be confirmed by metastable peaks in defocussed spectra.

Table 1. *Metastable ions in the H_2-CI-mass spectrum of di-O-acetyl-gomphoside* (**21**)

Found	567.5 ; 489	; 471.2 ; 444.3 ; 430–429.6		; 412	; 320	.
Calc.	567.54; 488.97; 471.15; 444.36; 429.70 and 429.62; 411.85; 319.91.					
Found	261	; 244.4 ; 97.3 ; 55.7 ; 50				.
Calc.	260.92; 244.23; 97.32; 55.70; 49.98.					



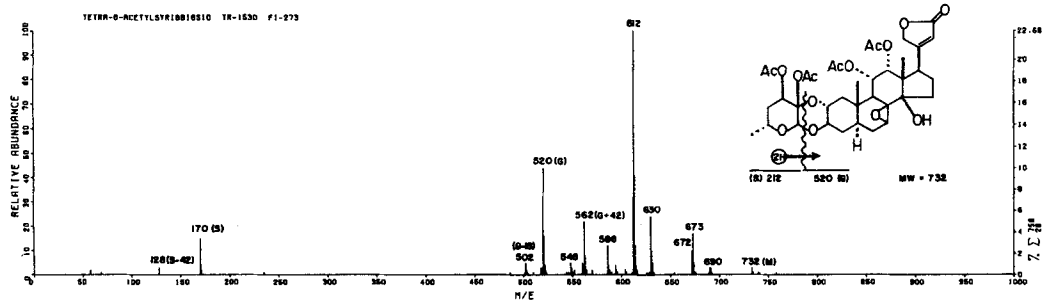


Fig. 9. *FI-mass spectrum of tetra-O-acetyl-syriobioside* (**8** = TR-1561), m.p. 338–340°, $C_{37}H_{48}O_{15}$ (732), probe temp. 260°. Assignments: 732 (*M*); 690 (*M* – 42), 673 (732–59); 672 (832–60); 630 (672–42); 612 (672–60); 594 (612–18); 586 (630–44); 562 (G + 42); 548 (?); 520 (G); 502 (G – 18); 170 (**43**).

[15]) Secured by Mr. *Derek Nelson*, Univ. of N.S.W. Australia on a *AEI-MS 902* instrument, H_2-pressure 0.3 Torr.

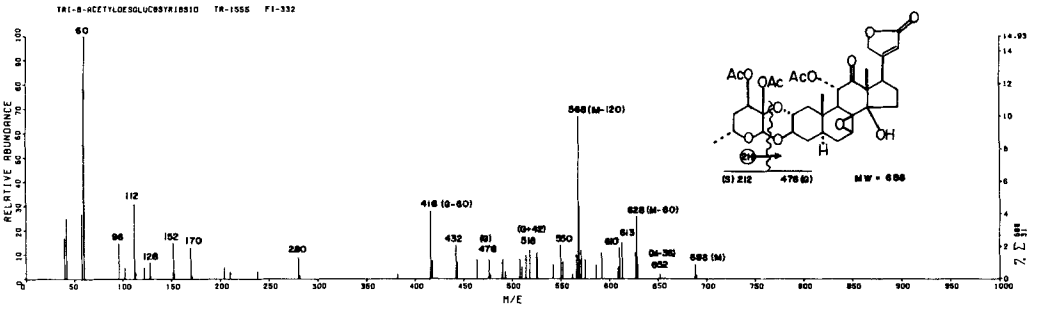


Fig. 10. *FI-mass spectrum of tri-*O*-acetyl-desgluco-syrioside* (**13** = TR-1555), m.p. 302–303°, $C_{35}H_{44}O_{14}$ (688) with hypothetical genin $C_{25}H_{32}O_9$ (476), probe temp. 270°. Assignments: 688 (*M*); 652 (688–18–18); 628 (688–60); 613 (628–15); 610 (628–18); 592 (610–18); 586 (628–42); 568 (628–60); 550 (568–18); 526 (568–42); 518 (G + 42); 508 (568–60); 490 (508–18); 476 (G); 464; 432 (476–44); 416 (G – 60); 280 (?); 170 (**43**); 152 (170–18); 128 (**18**).

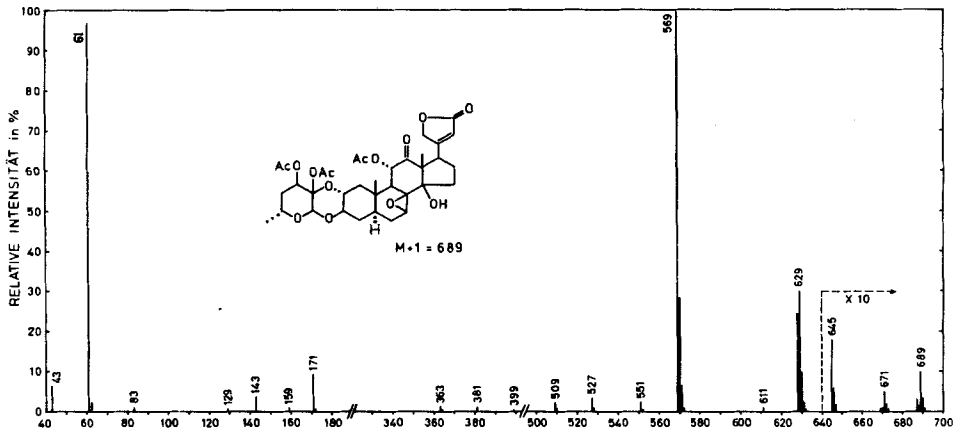
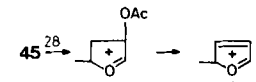


Fig. 11. *H_2-CI-mass spectrum*[15]) *of tri-*O*-acetyl-desgluco-syrioside* (**13** = TR-1555B), m.p. 302–303°, $C_{35}H_{34}O_{14}$ (688), source temp. 230°, probe temp. 190°. Assignments: 689 ($M+H^+$); 671 (689–18); 645 (689–44 or $M-1-42$); 629 (689–60), 611 (629–18 and 671–60); 587 (629–42); 569 (629–60); 551 (569–18 and 611–60); 527 (569–42 and 587–60); 509 (527–18, 551–42 and 569–60); 459 (G – 18 + H, R.A. < 1%); 399 (569–170 or 527–128, corr. to G – 60 – 18 + H^+); 381 (399–18 or 551–170 or 509–128); 363 (381–18); 171 (**45**); 129 (171–42); 143 (171–28); 83 (143–60). Peaks for G + H^+ (477) and mono-*O*-acetyl-G + H^+ (519) were absent. The ions at *m/e* 143 and 83 are probably derived from **45** and may best be represented by **51** and **52**. Observation of the key ion peaks at 689 ($M+H$); 629 ($M+1-60$); 569 ($M+1-60-60$) and 509 ($M+1-60-60-60$) provides strong evidence for the presence of 3 acetoxy groups in **13**.

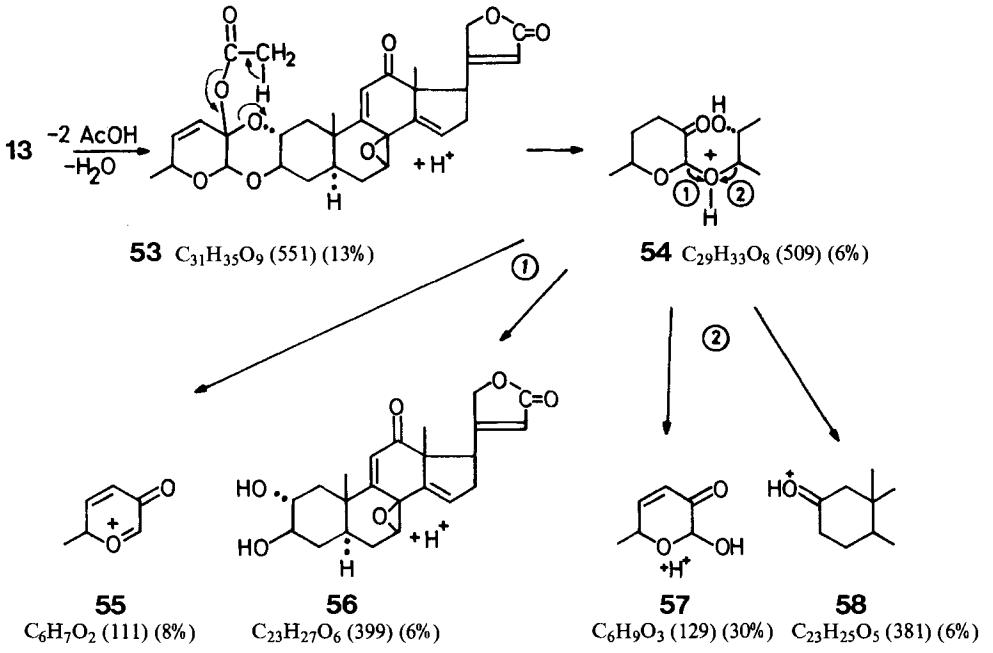


$C_7H_{11}O_3$ (143) C_5H_7O (83)

Table 1a. *Metastable ions in the H_2-CI-mass spectrum of tri-O-acetyl-desgluco-syrioside* (**13**)

Fragmentation (*m/e*)	*m** (found)	*m** (calc.)
689 $\xrightarrow{-18}$ 671	653.5	653.5
689 $\xrightarrow{-60}$ 629	574.2	574.2
629 $\xrightarrow{-60}$ 569	515	514.7
569 $\xrightarrow{-18}$ 551	533.5	533.6
551 $\xrightarrow{-42}$ 509	470	470.2

These ions indicate that at least one fragmentation sequence is:
689 ($M+H^+$) $\xrightarrow{-AcOH}$ 629 $\xrightarrow{-AcOH}$ 569 $\xrightarrow{-H_2O}$ 551 $\xrightarrow{-CH_2CO}$ 509 which can be interpreted as **13** → **54** and subsequent fragmentation in two ways: ① or ②.



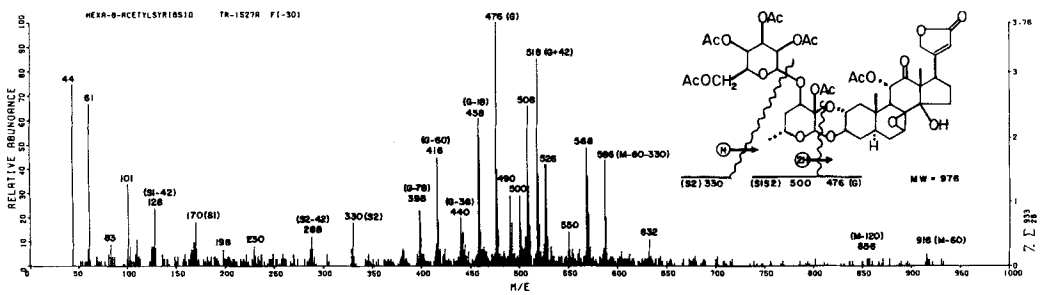


Fig. 12. *FI-mass spectrum of hexa-O-acetyl-syrioside* (**11** = TR-1527A), m.p. 192–193°, $C_{47}H_{60}O_{22}$ (976), probe temp. 265°. Assignments: $M+$ not visible; 916 ($M-60$); 856 (916–60); 632 (?); 586 (916–330); 568 (586–18); 550 (568–18); 526 (568–42); 518 (G+42); 508 (568–60); 500 (S1S2; 518–18); 490 (550–60); 476 (G); 458 (G – 18); 440 (G – 18 – 18); 416 (G – 60); 398 (416–18); 330 (S2); 288 (330–42); 170 (**43**); 128 (170–42); 110 (170–60; 128–18).

Table 2. *Compositions of ions by high resolution mass spectroscopy*[14])

Compound	Mol-Wt.	Fragment ion	Assignment
Syriobioside (**7**)	564	436: $C_{23}H_{32}O_8$	G
Desglucosyrioside (**12**)	562	416: $C_{23}H_{28}O_7$	G – 18
		398: $C_{23}H_{26}O_6$	416–18
		128: $C_6H_8O_3$	S(17)
Syrioside (**10**)	724	416: $C_{23}H_{28}O_7$	G – 18
Tetra-*O*-acetyl-syriobioside (**8**)	732	672: $C_{35}H_{44}O_{13}$	*M* – 60
		612: $C_{33}H_{40}O_{11}$	672–60
		562: $C_{29}H_{38}O_{11}$	G + 42
		170: $C_8H_{10}O_4$	S(**43**)
Hexa-*O*-acetyl-syrioside (**11**)	976	568: $C_{31}H_{36}O_{10}$	*M* – 333 – 60 – 18
		518: $C_{27}H_{34}O_{10}$	G + 42
		330: $C_{14}H_{18}O_9$	S2

Tri-*O*-acetyl-desgluco-syrioside Fragment ion	in H_2-CI-mass spectrum[15]) *m/e*	
	Found	Calc.
$C_{33}H_{41}O_{12}$ ($M+H^+-60$)[a])	629.2618	629.2597
$C_{33}H_{39}O_{11}$ (629–18)	611.2426	611.2492
$C_{31}H_{39}O_{11}$ (629–42)	587.2531	587.2492
$C_{31}H_{37}O_{10}$ (629–60)[a])	569.2418	569.2389
$C_{31}H_{35}O_9$ (569–18)[a])	551.2319	551.2281
$C_{29}H_{35}O_9$ (569–42)	527.2271	527.2281
$C_{29}H_{33}O_8$ (551–42)[a])	509.2223	509.2175
$C_{25}H_{31}O_8$ (G –18 + H^+) (R.A. < 3%)	459.2016	459.2019
$C_{23}H_{27}O_6$ (459–60)	399.1778	399.1807
$C_{23}H_{25}O_6$ (339–18)	381.1712	381.1702

[a]) Means metastable ions detected (see *Table 1a*).

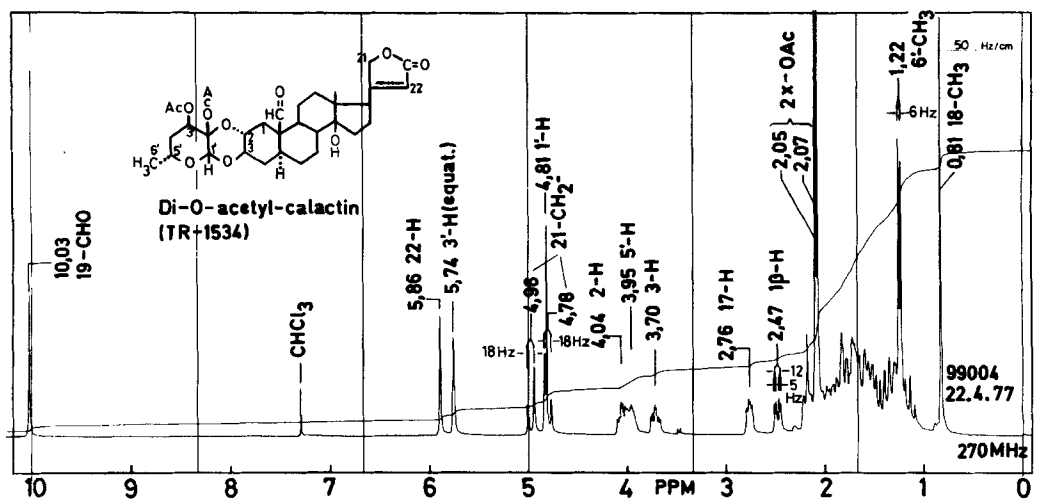


Fig. 13. *270-MHz-NMR. spectrum of di-*O*-acetyl-calactin* (**23** = TR-1534), m.p. 252–254° in $CDCl_3$[16]). Assignments tentative.

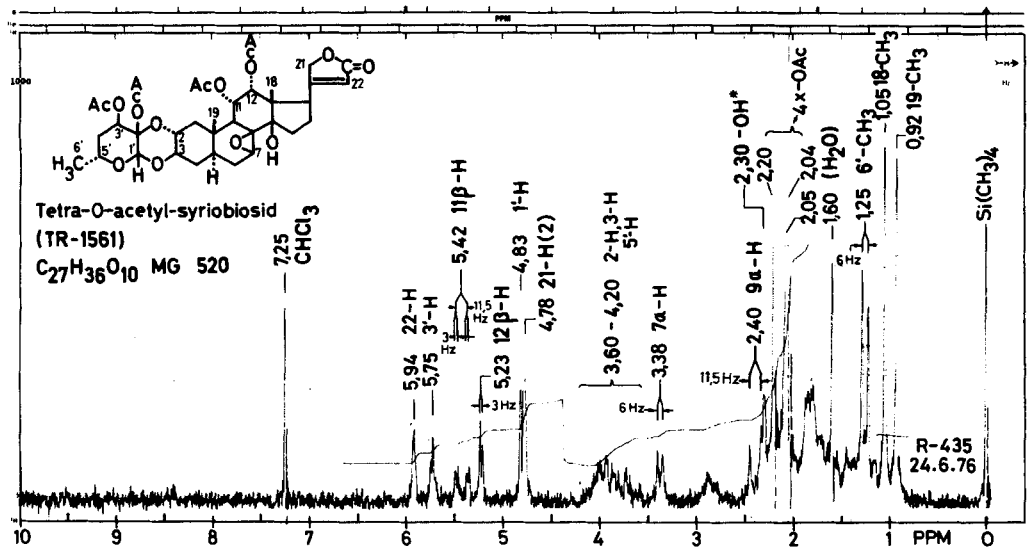


Fig. 14. *100-MHz-NMR. spectrum of tetra-O-acetyl-syriobioside* (**8** = TR-1561), m.p. 338–340° in $CDCl_3$[17]). Contained one mol water of crystallisation. Assignments tentative.

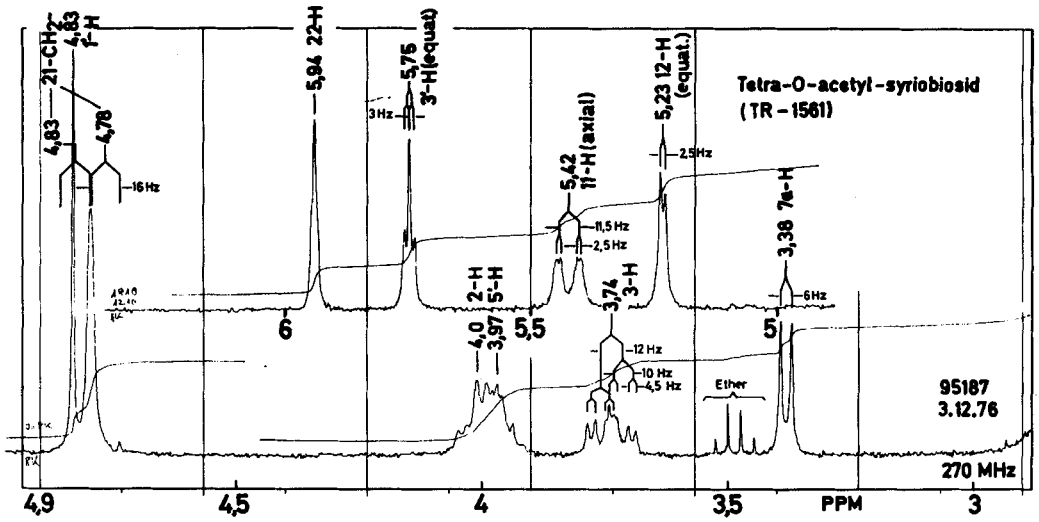


Fig. 15. *270-MHz-NMR. spectrum of tetra-O-acetyl-syriobioside* (**8**), same as *Figure 14*, but only showing lower field signals in higher resolution[16]).

[16]) We express our thanks to Dr. *W. Arnold*, Zentrale Forschungseinheiten *Roche Ltd.* Basel, for providing this spectrum and his help in interpretation. Performed on a *Bruker* HX 270 instrument with BNC 1180 computer.

[17]) We express our thanks to Dr. *H. Fuhrer* and Mr. *A. Borer*, Physics Laboratory *Ciba-Geigy Ltd.* Basel, for providing this spectrum and their help in interpretation. Performed on *Varian* spectrograph, model HA-100. The signals labelled at HO disappeared after shaking with D_2O.

Table 3. *Signals in ^{13}C-NMR. spectra in $CDCl_3$*

Carbon Number	Type	Di-*O*-acetyl-gomphoside[e] (**21**)	Di-*O*-acetyl-calactin[e] (**23**)	Tri-*O*-acetyl-desgluco-syroside (**13**)[18]	Di-*O*-acetyl-sarverogenin (**32**)[18]
1	CH_2	41.7	35.7	43.9	36.2[a]
2	CH–O– or CH_2	71.8[a][f]	70.8[a]	69.8[a]	25.5
3	CH–O–	71.1[a][f]	71.2[a]	70.9[a]	69.0
4	CH_2	32.0[b]	32.4[b]	31.5	32.0
5	CH	44.8	43.6	40.5	32.9
6	CH_2	27.7[c]	27.7[c]	35.6	35.5[a]
7	CH_2 or CH–O–	26.9[c]	26.9[c]	54.3	52.7
8	CH or C–O–	40.8	42.6	62.6	63.0
9	CH	49.6	48.6	45.2	32.9
10	$>C<$	38.0	52.8	37.7	34.4
11	CH_2 or CH–OAc	21.3	22.0	75.3	75.5
12	CH_2 or C=O	39.6	39.5	204.6	204.6
13	$>C<$	49.6	49.4	64.1	64.3
14	$>C$–OH	85.1	85.0	80.9	81.1
15	CH_2	32.9[b]	33.1[b]	28.4	28.5
16	CH_2	27.2[c]	27.4[c]	26.7	26.6
17	CH	50.8	50.7	41.8	41.8
18	CH_3	15.7	15.6	17.3	17.5
19	CH_3 or $C(=O)H$	13.7	206.4	13.7	23.0
20	$>C=$	174.9	174.2[d]	170.9	171.1
21	CH_2–O–	73.5	73.4	73.7	73.7
22	CH=	117.5	118.0	118.9	118.7
23	C=O	174.6[h]	173.9[d]	173.8	173.9
1′	$HC(O-)(O-)$	93.2	93.2	93.1	–
2′	$>C(O-)(O-)$	95.6	95.6	95.6	–
3′	CHOAc	70.3[g]	70.5[a]	70.3[a]	–
4′	CH_2	34.8	35.0	34.9	–
5′	CH–O–	66.4	66.6	66.7	–
6′	CH_3	20.9[d]	20.8	20.9[b]	–
acetyl	CH_3–CO	20.7[d]	20.8	20.7[b]	20.8
acetyl	CH_3–CO	21.7	21.6	21.8	21.4
acetyl	CH_3–CO	–	–	21.2	–
acetyl	CH_3–CO	168.8	168.5	169.7	169.5
acetyl	CH_3–CO	168.7	168.8	168.9	170.4
acetyl	CH_3–CO	–	–	168.3	–

[a][b][c][d] Signals within a vertical column may be reversed.

[e] Data from a number of derivatives of gomphoside and afroside (*H. T. A. Cheung* and *T. R. Watson*, unpublished results) provided the basis for assignments.

[f] Virtual coupling observed in single-frequency off-resonance spectra (sford) showing that the attached proton is tightly coupled vicinally.

[g] Sharp doublet in sford spectra, showing that the attached proton is loosely coupled vicinally.

[h] Doublet in sford spectra due to one J_{CCH}.

[18] We express our thanks to Dr. *H. Fuhrer* and Mr. *A. Borer*, Physics Laboratory *Ciba-Geigy Ltd.* Basel, for providing this spectrum and their help in interpretation. Performed on *Varian* spectrograph model XL-100-15 at 25.2 MHz.

Table 4. *Signals in ^1H-NMR. spectra in $CDCl_3$, assignments tentative*

Assignment (number of H)	Sp. Nr. (MHz)	C(18) (3)	C(19) (3) (1)	C(2) (1)	C(3) (1)	C(7) (1)	C(9) (1)	C(11) (1)	C(12) (1)	C(17) (1)	C(21) (2)	C(22) (1)
Di-*O*-acetyl-gomphoside (TRW and TR-1564) **(21)**	98159 (270)		0.844 (*s*) 0.878 (*s*)	3.84 (*m*) $J=13$ $J=\sim 10$ $J=4.5$	4.05 (*m*) $J=\sim 13$ $J=\sim 10$ $J=4.5$					2.78 ($d\times d$) $J=10$ $J=5$	4.89 (*AB*) $J=18$ $J=2$ $J=1.5$	5.88 (*s*)
Di-*O*-acetyl-sarverogenin (TR-1387) **(32)** [34]	22753 R-348 (100)	1.129 (*s*)	1.074 (*s*)		4.98	3.40 (*d*) $J=6$	2.54 (*d*) $J=13$	5.58 (*d*) $J=13$		3.88 (*t*) $J=8$ $J=8$	4.75 (*AB*)	5.92 (*s*)
Di-*O*-acetyl-calotropin (**25** or **26**) (TR-1533)	72970 R-440 (100)	0.82 (*s*)	10.01 (*s*) (CHO)	3.60–4.20 (*m*)							4.47 (*d*) 4.92 (*d*) $J(AB)=18$	5.86 (*s*)
Di-*O*-acetyl-calactin **(23)** (TR-1534) *(Fig. 11)*	99004 (270)	0.81 (*s*)	10.03 (*s*) (CHO)	3.7 (*m*) $J=13$ $J=\sim 10$ $J=4$	4.04 (*m*) $J=\sim 12$ $J=\sim 11$ $J=4$					2.76 ($d\times d$) $J=10$ $J=5$	4.78 (*q*) 4.96 (*q*) $J(AB)=18$ $J=2$ $J=1.5$	5.86 (*s*)
Tetra-*O*-acetyl-syriobioside (**8**) (TR-1561) *(Fig. 12* and *13)*	95187 (270)	1.048 (*s*)	0.921 (*s*)	3.74 (*m*) $J=12$ $J=10$ $J=4.5$	*ca.* 4.0 (*m*)	3.38 (*d*) $J=6$	2.40 (*d*) $J=11.5$	5.42 ($d\times d$) $J=11.5$ $J=2.5$	5.23 (*d*) $J=2.5$		4.78 4.83 $J(AB)=16$	5.94 (*s*)
Tri-*O*-acetyl-desglucosyrioside (**13**) (TR-1555-A) *(Fig. 14)*	72644 R-434 (100)	1.12 (*s*)	1.01 (*s*)	3.60–4.20 (*m*)		3.50 (*d*) $J=\sim 5$		5.66 (*d*) $J=11.5$			4.69 4.89 $J(AB)=18$	5.97 (*s*)
Hexa-*O*-acetyl-syrioside (**11**) (TR-1527)	94630 (270)	1.126 (*s*)	1.006 (*s*)	3.60–4.20 (*m*)		3.51 (*d*) $J=6.0$		5.68 (*d*) $J=11.5$			4.71 4.84 $J(AB)=18$ $J=2$ $J=1.5$	5.88 (*s*)

Table 4 (continued)

Assignment (number of H)	O–Ac genin	O–Ac sugar	O–Ac glucose	C(1′) (1)	C(3′) (1)	C(5′)	C(6′) (3)	C(1″) (1)	C(2″) (1)	C(3″) (1)	C(4″) (1)	C(5″) (1)	C(6″) (2)	Further signals
Di-*O*-acetyl-gomphoside (TRW and TR-1564) (**21**)		2.08 (*s*) 2.10 (*s*)		4.83 (*s*)	5.74 (*t*) $J=3$	*ca.* 3.97 (*m*)	1.24 (*d*) $J=6$							
Di-*O*-acetyl-sarverogenin (TR-1387) (**32**) [34]	2.0 (*s*) 2.15 (*s*)													
Di-*O*-acetyl-calotropin (**25** or **26**) (TR-1533)		2.03 (*s*) 2.09 (*s*)		5.54 (*s*)	5.80 (*qa*) $J(a,a)=10$ $J(a,e)=6$	3.60–4.20 (*m*)	1.26 (*d*) $J=6$							
Di-*O*-acetyl-calactin (**23**) (TR-1534) *(Fig. 11)*		2.06 (*s*) 2.08 (*s*)		4.80 (*s*)	5.74 (*qa*) $J(e,a)=3$ $J(e,e)=2.5$	*ca.* 3.95 (*m*)	1.22 (*d*) $J=6$							2.47 (*qa*) (1) equat.? $J=12$ $J=5$
Tetra-*O*-acetyl-syriobioside (**8**) (TR-1561) *(Fig. 12* and *13)*	2.07 (*s*) 2.20 (*s*)	2.05 (*s*) 2.07 (*s*)		4.83 (*s*)	5.75 (*t*) $J=3$	*ca.* 3.97 (*m*)	1.25 (*d*) $J=6$							
Tri-*O*-acetyl-desglucosyrioside (**13**) (TR-1555-A) *(Fig. 14)*	2.23 (*s*)	2.06 (*s*) 2.06 (*s*)		4.82 (*s*)	5.74 (*t*) covered	3.60–4.20 (*m*)	1.22 (*d*) $J=6$							
Hexa-*O*-acetyl-syrioside (**11**) (TR-1527)	2.22 (*s*)	2.06 (*s*)	2.01 (*s*) 2.01 (*s*) 2.02 (*s*) 2.10 (*s*)	4.71 (*s*)		3.60–4.20 (*m*)	1.21 (*d*) $J=6.5$	4.44 (*d*) $J=8$	5.01 (*t*) $J=8$ $J=9$	5.08 (*t*) $J=9$	5.13 (*t*) $J=9$	3.57–3.75 (*m*)	4.03 (*qa*) 4.35 (*qa*) $J(AB)=12$ $J=4.0$ $J=1.5$	2.34 (*qa*) $J=13$, $7=4$ 2.59 (*d*) $7=1.5$ (–OH)

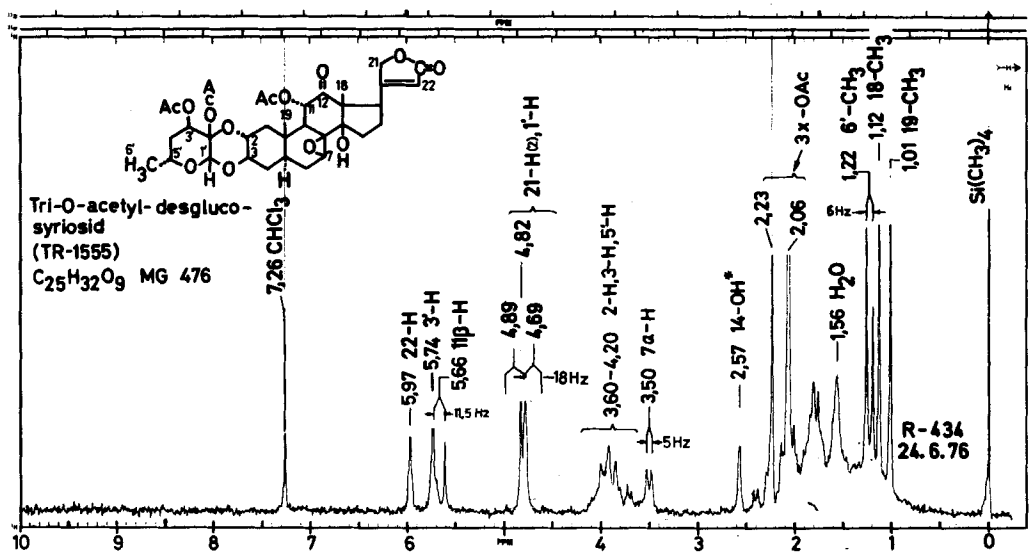


Fig. 16. *100-MHz-NMR. spectrum of tri-O-acetyl-desgluco-syrioside* (**13** = TR-1555-A), m.p. 300–301° (decomp.) in $CDCl_3$[17]), containing some water of crystallisation. Assignments tentative

Experimental Part

All m.p. were taken on the *'Kofler'* hot stage microscope and are corrected. Opt. rotation was measured on a *Schmidt & Haensch* polarimeter Nr. 11791 (visual), and a *Perkin Elmer* 141 polarimeter (photoelectric). Samples for combustion analyses were dried at 0.01 Torr. over P_2O_5 for 24 h at 20°. Analyses performed by Mr. *E. Thommen* on a *Perkin Elmer* CHN-analyzer Nr. 240 with amounts of 1.2–1.5 mg. Mass spectra in Arizona were produced by Mr. *Richard B. Scott*, using a *Varian* Atlas SMIB double focussing mass spectrometer with dual EI/FI ion source of the wire emitter type, maintained at 175°. For the low resolution (approx. 1500) FI spectra, anode and cathode voltages were +8 and −2 kV respectively, and the probe temperatures 235–270° (see *Figures*). The instrument was equipped with a digital mass indicator, and this was calibrated in the FI mode over the mass range 200–1000 using mixtures of phosphonitrilic compounds (*R.B. Scott & P. Brown*, unpubl.). For the high resolution (approx. 10,000; measurements in *Table 2*) EI was used together with slightly lower probe temperature. The computer program for plotting routine was written by Dr. *James J. Einck*.

Mass spectra in Sydney were produced by Mr. *Derek Nelson* of the School of Chemistry of the University of NSW, Australia, using an *MS*-9 double focussing mass spectrometer adapted for chemical ionisation. H_2 (0.3 Torr.) was used as the reagent gas. The source temperature was generally 220°, and the probe temperature 180–200°.

The following systems were used for PC.: *Whatman* No. 1 paper was impregnated with 33% its weight with formamide for systems A, B, C and with 34% its weight with water for system D. All PC. was descending flow.

Solvent A. Tetrahydrofurane/benzene/cyclohexane 1:3:6, saturated with formamide

Solvent B: Tetrahydrofurane/benzene/cyclohexane 1:2:3, saturated with formamide

These were used for *O*-acetyl-derivatives, which showed the following Rf-values.

Compound		System for PC.	
		A	B
		Rf-values	
Di-*O*-acetyl-gomphoside	(**21**)	0.38	0.51
Di-*O*-acetyl-calactin	(**23**)	0.12	0.26
Di-*O*-acetyl-calotropin	(**25** or **26**)	0.19	0.39
Tri-*O*-acetyl-desglucosyrioside	(**13**)	0.14	0.29
Tetra-*O*-acetyl-syrioside	(**8**)	0.15	0.30
Hexa-*O*-acetyl-syrioside	(**11**)	–	0.18

Solvent C: Tetrahydrofurane/benzene 1:1, saturated with formamide

Solvent D: Butanol/benzene 1:2, saturated with water (for paper impregnated with water)

These systems were used for free glycosides, system D particularly to differentiate syrioside (**10**).

Compound		System for PC.	
		C	D
		Rf-values	
Gomphoside	(**20**)	0.75	0.84
Calactin	(**22**)	0.56	0.83
Calotropin	(**24**)	0.34	0.82
Desglucosyrioside	(**12**)	0.33	0.76
Syriobioside	(7)	0.18	0.70
Syrioside	(**10**)	*ca.*0.04	0.28

Spots in PC. were visualized with *Kedde*'s reagent [41] or 2,2',4,4'-tetranitrodiphenyl [42] with semiquantitative estimation by comparison with known amounts of cardenolide.

TLC. was particularly convenient to differentiate desglucosyrioside (**12**) from syriobioside (**7**). The system used was silicagel '*Merck* HF 254+366 Type 60' and ethyl acetate as solvent, ascending. The solvent was allowed to flow a distance of 180 mm. The following values were found.

Compound		Running distance	Rf-values
Gomphoside	(**20**)	95 mm	0.53
Calactin	(**22**)	77 mm	0.43
Calotropin	(**24**)	58 mm	0.32
Desglucosyrioside	(**12**)	82 mm	0.45
Syrioside	(7)	*ca.* 5 mm	*ca.* 0.03

Spots in TLC. were visualized under UV. lamp, and by spraying with 15% *p*-toluenesulfonic acid in ethanol, and subsequent heating to 100–120°.

Checking for 'normal' sugars after vigorous hydrolysis (see [18], p. 1750). 1.5 mg glycoside was heated with 0.2 ml *Kiliani*-mixture [44][18a]) at 100° for 1 h, then evaporated *in vacuo* at 40°. The residue was dissolved in 0.2 ml water and again evaporated, and this process repeated once more. The final residue, dissolved in 0.1 ml water can be used directly for TLC. or PC. [17], or first extracted with chloroform [18]. The sugars were visualized by heating after spraying with *p*-aminohippuric acid + phthalic acid [45].

Checking for 2-deoxy sugars after mild hydrolysis (see [9], p. 945). 1.5 mg glycoside was dissolved in 0.2 ml methanol, and after addition of 0.2 ml 0.1N aqueous sulfuric acid boiled under reflux in a micro vessel for 30 min. The solution was then concentrated *in vacuo* to 0.1 ml, the same amount water added, and warmed again for 30 min at 60°. After cooling, the suspension was extracted with chloroform, the aqueous layer neutralized with freshly prepared pure $BaCO_3$ (precipitated from $Ba(OH)_2$ solution with CO_2, and washed with boiling water), filtered with suction through a little hardened paper, and the precipitate washed with a little hot water. The neutral solution and washings were evaporated

[18a]) 35 ml glacial acetic acid, 55 ml water and 10 ml conc. HCl-solution.

in vacuo and the residue checked for 2-deoxy sugar with xanthydrol reagent [46], or in TLC. and PC. [47], detection by heating after spraying with vanillin/perchloric acid to 110° [48]. In the case of syriobioside, and other chloroform soluble glycosides the chloroform extracts can be used for vigorous hydrolysis with *Kiliani*-mixture or for the *Hesse* osazone reaction.

Hesse's *osazone reaction* (see [19], p. 74) *in micro scale.* 2 mg calactin (or other compound of this type) are dissolved in 1 ml of freshly filtered saturated solution of 2,4-dinitrophenylhydrazine hydrochloride in 0.1N aqueous chlorhydric acid (= *ca.* 13 mg) by warming to 80°, and left for 2–3 days at 20° with occasional warming and shaking. In a positive reaction *ca.* 2–3 mg orange red precipitate is formed after this time, and *ca.* 0.9 additional mg may separate after standing for another 2 days. The precipitate is filtered off with suction, washed with 0.1N HCl and water, m.p. crude *ca.* 205–215°. With 5% KOH-solution in ethanol it gives a deep violet-blue solution.

Isolation of syrioside (**10**) *and syriobioside* (**7**). *Ca.* 2.5 kg (fresh weight) of roots of *Asclepias syriaca* (dug up in Oct. 1973) washed and dried at 45° gave 770 g of dry material. This was pulverized, extracted with 1.5 l boiling 50% aqueous methanol, filtered off with suction and the residue re-extracted 10 times with methanol/water mixtures of increasing methanol content, and pure methanol until the *Kedde*-reaction was negative. The combined extracts were extracted 3 times with petroleum ether. The petroleum ether layers were washed with water, dried over Na_2SO_4 and evaporated, giving *ca.* 10 g Pe-extract, *Kedde*-negative, which was discarded (fats *etc.*). The combined aqueous methanol layers were concentrated *in vacuo* to a volume of 250 ml, and the remaining aqueous suspension extracted 5 times with 500 ml ether, 400 ml chloroform and after half saturating with Na_2SO_4, with 300 ml chloroform/ethanol 3:2. The organic layers were washed in a counter current manner with water, 2N Na_2CO_3 and water. Before washing the chloroform/ethanol 3:2 layers, the aqueous washing medium was half saturated with Na_2SO_4. After drying, and evaporation the following crude extracts resulted:

ca. 12.000 g (extracted with ether)
8.195 g (extracted with chloroform)
3.895 g (extracted with chloroform/ethanol)

The ether-extract, according to PC. contained only a little of the fast running cardenolides, and was not further examined. The other two fractions were separated by column chromatography on SiO_2.

Investigation of the chloroform-extract. This material showed a positive *Kedde*-reaction and gave several spots in PC. and TLC. In spite of careful chromatography no pure cardenolides could yet be isolated.

Chromatography of chloroform/ethanol extracts. The 3.895 g of the product extracted by chloroform/ether were dissolved in 20 ml methanol mixed with 20 g SiO_2 ('*Merck*' 0.06–0.2 mm diameter), dried at 30° *in vacuo* and put on top of a column containing 260 g SiO_2, prepared in benzene. Elution was done with 300 ml solvent per fraction of benzene, benzene/ether and ether + 'mixture' (= chloroform/methanol/ethyl acetate 1:1:1).

Fr. 1–6 (108 mg eluted with benzene, benzene/ether 2:3 and ether + 8–20% 'mixture') were *Kedde*-negative, discarded.

Fr. 7–11 (260 mg eluted with ether + 30% 'mixture') showed on PC. four spots, the strongest corresponding to syriobioside.

Fr. 12 (107 mg eluted with same solvent) showed similar spots.

Fr. 13–19 (246 mg eluted with same solvent) gave from acetone/ether, 58 mg cryst. compound TR-1568, m.p. 99–100°, *Kedde*-negative, see below. The evaporated mother liquour (188 mg) gave in PC. (system C) three spots, and was rechromatographed for isolation of syriobioside (**7**); see below.

Fr. 20–26 (307 mg eluted with ether + 30–35% 'mixture') showed in PC. (system D) two very weak spots with Rf 0.71 and 0.82.

Fr. 27–31 (190 mg eluted with ether + 35–40% 'mixture') showed in PC. (system D) two spots with Rf 0.4 and 0.54.

Fr. 32–40 (990 mg eluted with ether + 40–50% 'mixture') gave from moist methanol/ether 268 mg syrioside (prep. TR-1524, m.p. 230–231°), and 40 mg second quality. The material from the mother liquours (589 mg) was used for enzymatic degradation.

Fr. 41–46 (355 mg eluted with ether + 50–60% 'mixture') gave in PC. (system D) a main spot like syrioside, which was used for enzymatic degradation.

Fr. 47–51 (270 mg eluted with ether + 60% 'mixture') gave in PC. (system D) four spots, which were also used for enzymatic degradation.

Fr. 52–57 (210 mg eluted with ether + 75% 'mixture') giving three very weak spots, were not further examined.

Fr. 58–64 (155 mg eluted with pure 'mixture') gave very weak spots, and were not further examined.

Isolation of syriobioside from non-fermented fractions. The 188 mg of non-crystalline material of fr. 13–19 from the main chromatogram were rechromatographed on 16 g SiO_2.

Fr. 1–13 (11 mg eluted with benzene, benzene/ether, ether and ether + 1–7% 'mixture') were *Kedde*-negative, and discarded.

Fr. 14–18 (7 mg eluted with ether + 8–10% 'mixture') gave a spot corresponding to desglucosyrioside (**12**), but no crystals. Used for acetylation.

Fr. 19–20 (3.5 mg eluted with ether + 11% 'mixture') gave two spots corresponding to **12** and **7**. Used for acetylation.

Fr. 21–24 (20.4 mg eluted with ether + 12–14% 'mixture') gave in PC. only the spot for syriobioside (**7**) and from methanol/ether 1.5 mg crystals as above, m.p. 305–307°, *Kedde*-negative. For separation of the amorphous material by preparative PC., see below.

Further fractions 25–57 gave 112 mg amorphous material showing only weak, slow moving spots in PC., not further examined.

The 19 mg amorphous material from fr. 21–24 were separated by prep. PC. on three sheets of *Whatman 3 MM* paper 19 cm wide impregnated with 33% of its weight with formamide, using solvent C. The solvent front had reached 39 cm after 3¼ h. The zone 100–150 mm from the start, containing syriobioside, was cut out, eluted with methanol/water under pressure, the solution evaporated *in vacuo* to 5 ml, slightly acidified with hydrochloric acid, and extracted 4 times with chloroform/ethanol 2:1. The extracts, washed as usual gave 10 mg crude material. This was liquified with a trace of methanol and ether added. The precipitate was washed with ether. The ether soluble part (1.9 mg) was *Kedde*-negative, and gave similar crystals as above, m.p. *ca.* 205° from methanol. The ether insoluble precipitate (8 mg) gave 2.5 mg crystalline syriobioside as colourless cubes from a trace of methanol + water after nucleation; this showed m.p. 215–220°, and $[\alpha]_D^{25} = +26.4° \pm 8°$ ($c = 0.26$, pyridine) after 5–10 min dropping to 0° after 30 min. According to PC. the material was unchanged after regeneration and gave similar crystals from water. These showed $[\alpha]_D^{23} = +10° \pm 5°$ ($c = 0.25$, $CHCl_3$) after 5 min, dropping to 0° again rather soon (after 10 min).

Enzymatic degradation of crude fractions. The amorphous material of fr. 32–40 (589 mg) combined with 311 mg material from fr. 41–46 (total 900 mg), was dissolved in 110 ml 1% aqueous acetic acid, 900 mg solid snail enzyme [43], and two drops toluene added. After thorough mixing it was left for 12 days at 30° with occasional shaking every day. It was then concentrated *in vacuo* at 40° to 10 ml, mixed with 200 ml abs. ethanol, warmed to 50°, and filtered through a very thin layer of 100 mg washed absorbent charcoal which was well washed again with ethanol. The mixed solutions were evaporated to 5 ml *in vacuo*, mixed with 5 ml water and extracted 4 times with 20 ml chloroform and after half saturating with Na_2SO_4 three times with chloroform/ethanol 3:2. The organic layers, washed, dried and evaporated gave: 375 mg chloroform soluble (PC. in system C showed a main spot for desglucosyrioside (**12**) and a weaker spot for syriobioside (**7**) and 212 mg chloroform/ethanol soluble material which showed only the spot for syriobioside (**7**) in PC. system C.

Chromatography of the chloroform soluble material from fermentation. The 375 mg material was chromatographed on a column of 30 g SiO_2 using 25 ml eluate from each fraction.

Fr. 1–5 eluted with benzene and benzene/ether gave no residue.

Fr. 6–13 (13 mg eluted with ether and ether + 2–10% 'mixture') gave in PC. (system C) a weak spot for desglucosyrioside (**12**) and three others having higher Rf-values. This material was used for acetylation.

Fr. 18–20 (30 mg eluted with ether + 11% 'mixture') gave in the PC. a strong spot for **12** and another spot of higher Rf-values; used for acetylation.

Fr. 21–26 (66 mg eluted with ether + 11 – 12% 'mixture') showed in the PC. (system C) a strong spot for desglucosyrioside (**12**) and a weak one for syriobioside (**7**) and gave from methanol/ether 2 mg crystals, m.p. 325–330°, *Kedde*-negative. The amorphous material from the mother liquour was rechromatographed, and gave 44.5 mg amorphous **12**, which was used for acetylation.

Fr. 27–33 (60 mg eluted with ether + 15–25% 'mixture') showed in PC. a very strong spot for syriobioside (**7**) and gave from methanol/water 11 mg cryst. syriobioside, m.p. 215–220°. The amorphous material from the mother liquour was used for acetylation.

Fr. 34–37 (26 mg eluted with ether + 25–35% 'mixture') showed in the PC. (system C) again the spot of syriobioside (**7**) and was used for acetylation.

Further fractions (65 mg eluted with ether + 40% 'mixture' and pure 'mixture') were *Kedde*-negative; discarded.

Chromatography of the chloroform/ethanol soluble material from fermentation. The 212 mg material were chromatographed on 15 g SiO_2 with 20 ml per fraction.

Fr. 1–8 (1.5 mg eluted with benzene, ether and ether + 12% 'mixture') were *Kedde*-negative; discarded.

Fr. 9–12 (8 mg eluted with ether + 13–17% 'mixture') gave in PC. (system C) three spots all running more quickly than syriobioside.

Fr. 14–24 (68 mg eluted with ether + 18–40% 'mixture') gave from methanol/ether *ca.* 1:6 16 mg cryst. syriobioside, m.p. 221–223°. The material from mother liquours slowly gave from water an additional 27 mg pure crystals, m.p. 224–226°.

Fr. 25–29 (29 mg eluted with ether + 45–80% 'mixture') contained only traces of syriobioside and gave two additional slow moving spots.

Fr. 30–37 (70 mg eluted with pure 'mixture') contained only traces of slow moving cardenolides.

Second batch. Roots dug up in Oct. 1974 washed dried and pulverized gave 820 g dry powder. This was extracted as for the first batch with ether (27 g), with chloroform (9.5 g) and with chloroform/ether 3:2 (5.9 g). The latter was chromatographed on 350 g SiO_2, for each fraction 500 ml of solvent were used.

Fr. 1–4 (685 mg eluted with benzene, ether and ether + 15% 'mixture') were *Kedde*-negative; discarded.

Fr. 5–7 (240 mg eluted with ether + 15–30% 'mixture') contained only a small amount of fast moving cardenolides.

Fr. 8–12 (440 mg eluted with ether + 30% 'mixture') gave in the PC. also only weak, fast moving spots.

Fr. 13–18 (400 mg eluted with ether + 30–40% 'mixture') gave in the PC. a long spot, similar to but slightly quicker than syriobioside.

Fr. 19–31 (1.35 g eluted with ether + 40–50% 'mixture') gave in PC. the spot of syrioside, but no crystals could be obtained.

Fr. 32–54 (1.1160 g eluted ether + 50% 'mixture' and pure 'mixture') contained no syrioside, but only little very highly polar cardenolides; discarded.

The fractions 19–31 (1.35 g) containing the syrioside were rechromatographed on 95 g SiO_2. The syrioside was concentrated in 890 mg of mixture from which no crystals could be got directly. This material was separated by partition chromatography using 250 g purified 'Hyflo-super Cel' (purified Kieselgur) impregnated with 250 ml water and moist benzene/butanol as the moving phase. The flow rate was 35–40 ml per hour, and each fraction *ca.* 120–160 ml, except the first and last ones which were 500–700 ml. The fractions 1–55 (390 mg eluted with benzene containing 0–20% butanol) contained no syrioside, only faster running material.

The fractions 56–62 (150 mg eluted with mixture containing 20–30% butanol) contained only syrioside and gave from methanol/ether 45 mg cryst. syrioside, m.p. 221–226°. Recrystallization from moist methanol/ether gave 35 mg pure material, m.p. 231–232°. The material from the mother liquour (115 mg) was used for enzymatic degradation to prepare desglucosyrioside.

The fractions 63–65 (43 mg eluted with benzene containing 30% butanol) was a mixture containing syrioside and a slower moving cardenolide (Rf = 0.24 in system E). This material was also used for enzymatic degradation.

Fr. 66–80 (125 mg eluted with benzene containing 30% butanol) showed only the spot with Rf 0.24 (system E); discarded.

Syrioside (**10**) (TR-1524) colourless needles as hydrate from moist methanol/ether, m.p. 230–231° (highly viscous melt), $[\alpha]_D^{23} = -12.8° \pm 2°$ (pyridine). – UV.: $\lambda_{max}^{eth.} = 215$ nm ($\log\varepsilon = 4.22$) and shoulder at *ca.* 270–290 nm ($\log\varepsilon$ at 280 *ca.* 2.0). – IR.: 1782; 1744; 1626 (butenolide); 1705 (keto-group) *Figure 1.* – Mass spectrum see *Figure 4.* – After vigorous hydrolysis with *Kiliani*-mixture only glucose was obtained as normal sugar. Mixed m.p. with authentic material gave no depression, and running distances in PC. and TLC. were the same. $C_{35}H_{48}O_{16} + H_2O$ (742.75) Calc. C 56.59 H 6.78% Found C 56.55 H 6.88%, no ash. *Masler et al.* [3a] found m.p. 234–237°, $[\alpha]_D^{23} = -13.6°$ ($c = 1.3$, pyridine) and C 55.06 H 7.30% corresponding to a dihydrate $C_{35}H_{48}O_{16} + 2\,H_2O$ (760.77) calc. C 55.25 H 6.89%.

Hexa-O-acetyl-syrioside (**11**) (TR-1527). 94 mg syrioside, 0.8 ml acetic anhydride and 1.2 ml abs. pyridine were sealed *in vacuo* and kept for 8 days at 35°. The mixture was evaporated *in vacuo* at 50°, the residue dissolved in chloroform, washed with dilute hydrochloric acid, $KHCO_3$-solution and water, dried over Na_2SO_4 and evaporated *in vacuo*. The residue (130 mg) gave crystals from methanol/ether, m.p. 192–193° (viscous melt). For securing really pure material, the product was chromatographed on 7 g SiO_2. Benzene, ether and ether with 1–2% 'mixture' eluted only traces of material (discarded). The fractions 12–16 (122 mg eluted with ether + 4–45% 'mixture') showed only one spot in the PC. The following fractions gave only little material and were discarded. The mentioned fractions 12–16 gave from chloroform/ether colourless leaflets, m.p. 205–206° (viscous melt, very slow heating gave m.p. *ca.* 192–193°), $[\alpha]_D^{24} = -1.2° \pm 2°$ ($c = 1$, $CHCl_3$); for analysis recrystallized from methanol/ether (TR-1527-C). These crystals contained water but no chloroform and no ash. After careful drying *in vacuo* the residue was hygroscopic and had to be introduced with appropriate care into the analyser.

$C_{47}H_{60}O_{22} + H_2O$ (994.97) Calc. C 56.73 H 6.28% Found C 56.43 H 6.00%

Mixed m.p. with authentic material [3b] gave no depression, running distances in PC. were identical. *Masler et al.* [3b] found m.p. 185–189°, $[\alpha]_D^{22} = -2.5°$ ($c = 0.99$, $CHCl_3$) and C 56.87 H 6.47%.

Syriobioside (**7**) (TR-1525) colourless rectangular crystals from water or needles from methanol/ether, m.p. 221–223° (viscous melt), containing water but no ash. – UV.: $\lambda_{max}^{eth.} = 217$ nm ($\log\varepsilon = 4.14$). – IR. (KBr): 1778; 1737; 1622 (butenolide); no peak or shoulder of a keto-group was visible at *ca.* 1710–1720 cm^{-1}. – ^{1}H-NMR. (R-429 in $CDCl_3$): 0.895 (*s*, 3, 3 H–C(19)); 1.09 (*s*, 3, 3 H–C(18)); 4.80 (*s*, 1, H–C(1′)); 4.85 (*d*, 2, $J = 2$, 2 H–C(21)); 5.90 (*s*, 1, H–C(22)). – Mass spectrum see *Figure 4*.

No 2-deoxysugar was detectable after mild hydrolysis and no 'normal' sugar after vigorous hydrolysis with *Kiliani*-mixture, but *Hesse*'s osazone reaction was strongly positive.

$C_{29}H_{40}O_{11} + H_2O$ (582.63) Calc. C 59.77 H 7.27% Found C 59.80 H 7.35%

Mixed m.p. with authentic material gave no depression, running properties in TLC. and PC. were the same. *Masler et al.* [3a] found m.p. 220–222°, $[\alpha]_D^{22} = +11.5°$ (pyridine), C 60.15 H 7.79%. We observed mutarotation (see above).

Tetra-O-acetyl-syriobioside (**8**) (TR-1561). 31 mg syriobioside (**7**), second quality, 0.6 ml abs. pyridine and 0.4 ml acetic anhydride were treated as described for **11**. The crude product (42 mg) gave crystals from acetone/ether, m.p. 300–306°. To eliminate impurities the whole material (42 mg) was chromatographed on 1.7 g Al_2O_3 (*Woelm*, neutral, activity IV), fractions of 5 ml each being taken. Elution with benzene, benzene/ether and pure ether (all with 1% ethyl acetate to prevent deacetylation) gave only *ca.* 4 mg *Kedde*-negative material (discarded). The fractions 8–13 eluted with ether + 2–8% 'mixture' contained the bulk of **8**. Further fractions, eluted with ether + 14–20% 'mixture' still contained a little **8**, and more polar material. After reacetylation and chromatography they also gave a little pure **8**. From methanol/ether colourless leaflets, m.p. 338–340° (decomp.); $[\alpha]_D^{15} = +33.2° \pm 3°$ ($c = 0.59$, $CHCl_3$); containing water but no ash. – UV.: $\lambda_{max}^{eth.} = 213$ nm ($\log\varepsilon = 4.23$) no shoulder visible between 270–290 nm. – ^{1}H-NMR. spectrum see *Figures 14* and *15*. – Mass spectrum see *Figure 9*.

$C_{37}H_{48}O_{15} + H_2O$ (750.77) Calc. C 59.19 H 6.71% Found C 59.33 H 6.53%

Enzymatic degradation of syrioside. 50 mg pure syrioside (**10**), m.p. 230–231° and 50 mg snail enzyme were dissolved in 7.5 ml 1% aqueous acetic acid, two drops of toluene added, and left closed at 30° for 12 days with occasional shaking. Working up as mentioned above gave 43.5 mg chloroform soluble material (crude **12**) and 10 mg water soluble sirup. The latter showed in PC. and TLC. only the spot of glucose.

The chloroform soluble part (43.5 mg) was chromatographed on 3.5 g SiO_2. The first 12 fractions (eluted with benzene, ether and ether + 1–8% 'mixture') gave 2.3 mg *Kedde*-negative material (impurities from enzyme; discarded).

Fr. 13–15 (6 mg, eluted with ether + 10–12% 'mixture') consisted of nearly pure desglucosyrioside (**12**) still containing traces of two faster running cardenolides.

Fr. 16–21 (23 mg, eluted with ether + 12% 'mixture') gave only the spot of **12** in PC.

Further fractions 22–25 (eluted with ether + 14–30% 'mixture') gave only 1.7 mg amorphous material giving only three weak spots in PC. (discarded).

Desglucosyrioside (**12**) (TR-1554). Slowly gave crystals from slightly moist chloroform/ether, m.p. 203–204° (viscous melt), $[\alpha]_D^{22} = +25.5° \pm 1°$ ($c = 1.2$, $CHCl_3$). Mixed m.p. with syriobioside gave no distinct depression. – IR. (KBr): (see *Fig. 2*) showes ketoband at 1709 cm^{-1}. – ^{1}H-NMR. (R-436 in

$CDCl_3$): 1.06 (*s*, 3, 3 H–C(19)); 1.19 (*s*, 3, 3 H–C(18)); 1.23 (*d*, $J=6$, 3, 3 H–C(6')); 3.45 (*d*, $J=5$, 1, H–C(7)); *ca.* 4.8 (*s*, C(1'), partly covered by C(21)); 5.99 (*s*, 1, H–C(22)). – MS.: see *Figure 5*.

$C_{29}H_{38}O_{11}+H_2O$ (598.62) Calc. C 58.18 H 7.07% Found C 58.02 H 6.66%

Tri-O-acetyl-desglucosyrioside (**13**) (TR-1555) *from pure* **12**. 14 mg desglucosyrioside (**12**), 0.3 ml abs. pyridine and 0.2 ml acetic anhydride were treated as described for **11**. The crude product (18 mg) was purified by chromatography on 800 mg Al_2O_3 (*Woelm*, neutral activity IV). The column prepared with benzene containing 1% ethyl acetate (to prevent deacetylation), was eluted (1.5 ml per fraction) with benzene/ether (+1% ethyl acetate), ether (+1% ethyl acetate) and ether + increasing amount of 'mixture'. The pure compound **13** (15 mg) was eluted with ether + 7–20% 'mixture'. Colourless leaflets from dry methanol/ether, water and ash-free, m.p. 302–303°, $[\alpha]_D^{18}=+3.4°\pm2°$ ($c=1$, $CHCl_3$), mixed m.p. with tetra-*O*-acetyl-syriobioside (**8**) gave no distinct depression. – UV.: $\lambda_{max}^{eth.}=213$ nm with shoulder between 270–295 nm, ($\log\varepsilon=2.34$ at 283 nm). – ^{1}H- and ^{13}C-NMR.: see *Figure 16* and *Tables 3* and *4*. – MS.: see *Figures 10* and *11*.

$C_{35}H_{44}O_{14}$ (688.70) Calc. C 61.03 H 6.44% Found C 60.84 H 6.56%

An additional quantity of *ca.* 15 mg pure **13** (m.p. 301–303°) could be obtained from the combined amorphous fractions containing **12** after acetylation and chromatography on Al_2O_3.

Di-O-acetyl calactin (**23** or **23A**) (TR-1534) *from pure calactin.* 10 mg pure calactin (**22** or **22A**), m.p. 266–267° (dec.) giving only one spot in PC. (system C), 0.15 ml abs. pyridine and 0.1 ml acetic anhydride were treated as described for **11**. The crude product (13 mg) gave colourless crystals arranged in spherical clusters, m.p. 252–254°, $[\alpha]_D^{22}=+42.3°\pm2°$ (after 2 min) dropping to $+38.1°\pm2°$ (after six min, constant), ($c=0.84$, $CHCl_3$). – ^{1}H-NMR. (R-429 in $CDCl_3$)[19]): see *Table 4*. – EI-MS. (Nr. 17216, 30.1.75, probe temp. 100°[20])) gave no molecular peak ($M^+=616$) but peaks (relative intensity calc. for *m/e* 497 = 100%) at *m/e* 573 (6%, $M-43$); 556 (84%, $M-60$); 538 (9%, 556–18); 497 (100%, $M-60-60+1$); 479 (16%, 497–18); 478 (22%, $M-60-60-18$); neither G+42 (446) nor G (404) was visible but 387 (10%, G–18+1); 369 (15%, G–18–18+1) and 351 (16%, 369–18); 341 (18%, 369–28); 323 (33%, 341–18 and 351–28); 269 (10%); 171 (45%, **41**+H=**43**); 129 (20%, **18**+H); 117 (18%) and 111 (18%, 129–18).

Di-O-acetyl-calactin (**23** or **23A**) (TR-1534) *and Di-O-acetyl-calotropin* (**25** or **25A** or **26**) (TR-1533) *from mixtures.* 12 mg cryst. mixture of calotropin (**14** or **24A**) + calactin (**22**) from *Pergularia extensa* [49] (rich in **24**) was acetylated as described above. The crude product (16 mg) was separated by preparative PC. on three sheets of *Whatman 3 MM* paper 19 cm wide impregnated with 33% of formamide using solvent B. The front at 39 cm was reached in about 4 h. Small strips were cut out for spraying with *Kedde*'s reagent. Positive reaction was observed in 4 zones. Each was cut out, eluted with methanol/water 95:5, the solution evaporated *in vacuo*, the residue dissolved in chloroform/ether, washed with 2N HCl, water, 2N Na_2CO_3 and water, dried over Na_2SO_4 and evaporated.

3 mg zone 1 (0–45 mm from start) contained slow mowing material; discarded.

3 mg zone 2 (120–165 mm from start) contained di-*O*-acetyl-calactin (**23**) which crystallized.

8 mg zone 3 (180–225 mm from start) gave only the spot of di-*O*-acetyl-calotropin (by comparison with material obtained from 1 mg of pure calotropin by acetylation.

1.5 mg zone 4 (290–320 mm from start) contained an unknown compound (TR-1562) which ran about three times faster than di-*O*-acetyl-calotropin in system A, *i.e.* only 0.75 as far as *O*-acetyl-uzarigenin (**1**, R = Ac). Not further investigated.

The material from each of the zone 2–4 was purified by chromatography on SiO_2 or Al_2O_3 (*Woelm*, neutral, activity III), or both to eliminate impurities and products of autoxidation.

The 3 mg of zone 2 were combined with 33 mg of similar material from other experiments (total 36 mg), and chromatographed on 2.5 g Al_2O_3 using 3 ml solvent per fraction. The column was prepared with benzene + 1% ethyl acetate to prevent deacetylation and the same amount was added to all eluents. Fractions 1–8 (9 mg eluted with benzene, ether and ether + 1–4% 'mixture') were *Kedde*-negative; discarded.

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[19]) Performed on a '*Varian* spectrophotometer, model HA-100'. We are very grateful to Dr. *H. Fuhrer* and Mr. *A. Borer*, Physiklaboratorium *Ciba-Geigy AG* Basel, for providing this spectrum.

[20]) Performed on a '*Varian-CH*$_7$' instrument with direct inlet system, at 70 eV and 300 u Amp. We thank Dr. *H. Hürzeler*, Physiklaboratorium *Ciba-Geigy AG* Basel, for providing this spectrum.

Fr. 10–18 (11.2 mg eluted with ether + 6–30% 'mixture') showed in PC. only the spot of **23**, and from methanol/ether gave 6 mg crystalline di-*O*-acetyl-calactin (**23**), m.p. 262–264°.

Fr. 19–26 (3 mg, eluted with ether + 'mixture' and pure 'mixture') gave only a very weak spot at start; discarded.

The 8 mg of zone 3 were combined with 25 mg of similar material from other experiments. These 33 mg were first chromatographed on 2 g SiO_2. 8.5 mg of *Kedde*-negative material obtained from the first 10 fractions was discarded. The di-*O*-acetyl-calotropin (**25** or **26**) came down in fractions 11–16 (10.1 mg, eluted with ether + 10–30% 'mixture'). Further fractions 17–28 gave 7.3 mg impurities; discarded.

The mentioned 10.1 mg concentrate was rechromatographed on 1 g Al_2O_3 (*Woelm*, neutral, activity III), the column being prepared with benzene containing 1% ethyl acetate to prevent deacetylation. Here again 0.7 mg *Kedde*-negative material obtained from the first six fractions was discarded. Purest di-*O*-acetyl-calotropin (**25** or **26**) (5.4 mg) was eluted with ether + 4–8% 'mixture', and 1 mg of second quality material in the two following fractions (10–12% 'mixture'). The last fractions gave only 2 mg of impurities.

Di-O-acetyl-calotropin (**25** or **25A** or **26**) (TR-1533). The above 5.4 mg were the purest material obtained as amorphous glass, $[\alpha]_D^{17} = 0° \pm 2°$ ($c = 0.6$, $CHCl_3$). – ^{1}H-NMR. (R-440 in $CDCl_3$)[19], see *Table 4*. – In EI mass spectrum (Nr. 20528, 19.11.76, probe temp. 160°)[20], no molecular peak ($M+ = 616$) visible but strong peak at *m/e* 556 (100%, $M-60$); 497 (100%, $M-60-60+1$); neither G + 42 (446) nor G (404) was visible but 387 (76% = G – 18 + 1); 369 (48% = 387–18); 351 (40% = 369–18); 341 (43% = 369–28); 323 (53% = 351–28 and 341–18); 269 (24%); 171 (78% = **43**); 129 (96% = **18** + H); 128 (92% = **18**); 111 (84% = 129–18).

Acetolysis of hexa-O-acetyl-syrioside (**11**). (Conditions see [50]). 102 mg hexa-*O*-acetyl-syrioside (**11**) were dissolved in 1 ml acetic anhydride, 25 mg anhydrous $ZnCl_2$ (freshly fused *in vacuo*) was added, and the mixture heated to 100° for 30 min. Then it was poured on 10 g broken ice and left for 15 h at 20°. Extraction (three times with 6 ml chloroform each time) washing of the solutions with water, 2N Na_2CO_3 and water, drying and evaporation gave 92 mg crude brown product. This was chromatographed on 5.5 g SiO_2 with 10 ml per fraction.

The first four fractions (eluted with benzene and benzene/ether mixtures gave only I mg amorphous material; discarded.

Fractions 5–7 (26 mg eluted with pure ether) gave from benzene/petrolether 13 mg crystalline penta-*O*-acetyl-D-glucose TR-1528), m.p. 96–103°, $[\alpha]_D^{24} = +64.5° \pm 1°$ ($c = 1.3$, $CHCl_3$).

$C_{16}H_{22}O_{11}$ (390.34) Calc. C 49.23 H 5.68% Found C 49.79 H 5.90%

Further fractions (eluted with ether + 'mixture' and pure 'mixture') gave 66 mg amorphous material, and no crystals could be obtained after reacetylation and further chromatography. No indication for the presence of octa-*O*-acetyl-gentiobiose was found which is formed under our conditions of acetolysis from glycosides containing this disaccharide [50].

The 13 mg of crystals (TR-1528) were a mixture of 61.2% penta-*O*-acetyl-*α*-D-glucose with 38.8% *β*-derivative. These two isomers could not be separated in normal chromatography on SiO_2 and gave the same Rf-values in TLC. An artifical mixture of 62% penta-*O*-acetyl-*α*-D-glucose (m.p. 111–112°, $[\alpha]_D^{20} = +101.6°$, $CHCl_3$) with 38% *β*-derivative (m.p. 130–131° $[\alpha]_D^{20} = +3.8°$, $CHCl_3$) gave crystals from benzene/petrolether with m.p. 96–103°, $[\alpha]_D^{20} = +67.3° \pm 1°$ ($c = 1.37$, $CHCl_3$). The mixture with TR-1528 gave no depression and the running distance on TLC. was exactly the same.

REFERENCES

[1] *R. E. Woodson, jr.*, The North American species of *Asclepias* L., Ann. Missouri Bot. Gard. *41*, 1 (1954).

[2] *Š. Bauer, L. Masler, O. Bauerová & D. Šikl*, Experientia *17*, 15 (1961) and references on former work given therein.

[3] *L. Masler, Š. Bauer, O. Bauerová & D. Šikl*, Coll. Czechoslov. chem. Commun. *27*, 872 (1962); *idem*, ibid *27*, 895 (1962).

[4] *J. Patričić*, Farm. Glas. *23*, 3 (1967) Chem. Abstr. *67*, 8686 g.

[5] *B. Singh & R. P. Rastogi*, Review article: Cardenolides-glucosides and genins, Phytochemistry *9*, 315–331 (1970).

[6] a) *H. Mitsuhashi, K. Hayashi & K. Tomimoto*, Chem. pharm. Bull. Japan *18*, 828 (1970); b) *V. Pápay, L. Tóth, I. Novák & M. Fakas*, Herba Hungarica *12*, 115 (1973).

[7] *S. S. Duffey & G. G. E. Scudder,* J. Insect. Physiol. *18,* 63 (1972).
[8] *C. N. Roeske, J. N. Seiber, L. P. Brower & C. M. Moffitt,* Rec. Adv. in Phytochemistry *10,* 93–167 (1976).
[9] *S. Rangaswami & T. Reichstein,* Helv. *32,* 939 (1949).
[10] *F. Brüschweiler, W. Stöcklin, K. Stöckel & T. Reichstein,* Helv. *52,* 2086 (1969).
[11] *C. Casagrande, F. Ronchetti & G. Russo,* Tetrahedron *30,* 3587 (1974).
[12] *M. Okada & T. Anjyo,* Chem. pharm. Bull. Japan *23,* 2039 (1975).
[13] *T. Reichstein,* Naturwiss. Rundschau *20* (12), 499 (1967); *T. Reichstein, J. von Euw, J. A. Parsons & M. Rothschild,* Science *161,* 861 (1968); *M. Rothschild, J. von Euw, T. Reichstein, D. A. S. Smith & J. Pierre,* Proc. Roy. Soc. London B *90,* 1 (1975).
[14] *L. P. Brower,* Scientific American *220* (2), 22 (1969).
[15] *C. Mannich & G. Siewert,* Ber. Deutsch. chem. Ges. *75,* 737 (1942).
[16] *A. Stoll, A. Hofmann & W. Kreis,* Zschr. f. Physiol. Chem. *235,* 249 (1935).
[17] *H. Kaufmann, P. Mühlradt & T. Reichstein,* Helv. *50,* 2287 (1967).
[18] *P. R. O. Bally, K. Mohr & T. Reichstein,* Helv. *34,* 1740 (1951).
[19] *G. Hesse, F. Reicheneder & H. Eysenbach,* Liebigs Ann. Chem. *537,* 67 (1939).
[20] a) *T. R. Watson & S. E. Wright,* Chemistry & Ind. *1954,* 1178; b) Austral. J. Chemistry *9,* 497 (1956); c) ibid *10,* 79 (1957).
[21] *R. G. Coombe & T. R. Watson,* Austral. J. Chemistry *17,* 92 (1964).
[22] *R. M. Carman, R. G. Coombe & T. R. Watson,* Austral. J. Chemistry *17,* 573 (1964).
[23] *F. Brüschweiler, K. Stöckel & T. Reichstein,* Helv. *52,* 2276 (1969).
[24] *G. Hesse,* Angew. Chem. *61,* 339 (1949).
[25] *G. Hesse, L. J. Heuser, E. Hütz & R. Reicheneder,* Liebigs Ann. Chem. *566,* 130 (1950).
[26] *G. Hesse & H. Hertel,* Angew. Chem. *69,* 61 (1957).
[27] *G. Hesse, H. Fasolt & W. Geiger,* Liebigs Ann. Chem. *625,* 157 (1959).
[28] *G. Hesse, H. Hertel & K. Mix,* Liebigs Ann. Chem. *625,* 174 (1959).
[29] *D. H. G. Crout, R. F. Curtis, C. H. Hassall & T. L. Jones,* Tetrahedron Letters *2,* 63 (1963).
[30] *D. H. G. Crout, C. H. Hassall & T. L. Jones,* J. chem. Soc. *1964,* 2187.
[31] *T. Reichstein,* Naturwiss. Rundschau *20,* 499 (1967).
[32] a) *P. Brown, F. Brüschweiler, G. R. Petit & T. Reichstein,* J. Amer. chem. Soc. *92,* 4470 (1970); b) idem, Org. Mass Spectrom. *5,* 573 (1971).
[33] *A. H. Jackson,* Endeavour (new ser.) *1* (2), 75 (1977).
[34] *K. Saameli,* Helv. Physiol. Acta *25,* CR-219 (1967).
[35] *K. R. H. Repke & H. J. Portius,* Planta Med. Suppl. *4,* 66–78 (1971).
[36] *B. Singh & R. P. Rastogi,* Phytochemistry *11,* 757 (1972).
[37] *H. Fuhrer, R. F. Zürcher & T. Reichstein,* Helv. *52,* 616 (1969).
[38] a) *R. F. Curtis, C. H. Hassall & J. Weatherston,* J. Chem. Soc. *1962,* 4225; b) *D. H. G. Crout, R. F. Curtis & C. H. Hassall,* ibid *1962,* 1866.
[39] *W. Klyne,* Biochem. J. *47,* xli (1950).
[40] a) *B. Birdsall, N. J. M. Birdsall & J. Feeney,* Chem. Commun. *1972,* 312; b) *Pretsch, Clerc, Seibl & Simon:* «Tabellen zur Strukturaufklärung organischer Verbindungen mit spektroskopischen Methoden», Springer, Berlin-Heidelberg-New York 1976.
[41] *D. L. Kedde,* Pharmac. Weekbl. *82,* 741 (1947).
[42] *R. Mauli, Ch. Tamm & T. Reichstein,* Helv. *40,* 284 (particul. 293) (1957).
[43] *H. Huber, F. Blindenbacher, K. Mohr, P. Speiser & T. Reichstein,* Helv. *34,* 46 (1951).
[44] *H. Kiliani,* Ber. Deutsch. chem. Ges. *63,* 2866 (1930).
[45] *F. Scheffer & R. Kikuth,* Z. Analyt. Chem. *191,* 116 (1962).
[46] *M. M. Pesez,* Ann. pharmac. franç. *10,* 104 (1952), see also *E. Abisch & T. Reichstein,* Helv. *43,* 1845 (1960) part. p. 1860.
[47] a) *O. Renkonen & O. Schindler,* Helv. *39,* 1490 (1956); b) *R. Tschesche & K.-H. Bratge,* Chem. Ber. *85,* 1042 (1952).
[48] *A. P. Mac. Lennan, H. M. Randall & D. W. Smith,* Analyt. Chemistry *31,* 2020 (1959).
[49] *O. P. Mittal, Ch. Tamm & T. Reichstein,* Helv. *45,* 907 (1962).
[50] a) *R. Tschesche,* Ber. Deutsch. chem. Ges. *69,* 2368 (1936); b) *A. Rheiner, A. Hunger & T. Reichstein,* Helv. *35,* 687 (1952); c) *P. Hauschild-Rogat, J. v. Euw, O. Schindler, Ek. Weiss & T. Reichstein,* Helv. *40,* 2116 (1962).
[51] *Tollens-Elsner,* «Kurzes Handbuch der Kohlehydrate», 4. Aufl. Leipzig 1935, p. 211 and see particul. *C. S. Hudson & J. K. Dale,* J. Amer. Chem. Soc. *37,* 1264 (1915).