DEEPER INSIGHTS INTO THE STEREOSTRUCTURE OF STRICTOSAMIDE TETRAACETATE AND METHYLISOALANGISIDE TETRAACETATE, THE KEY REFERENCE MOLECULES IN MONOTERPENOID INDOLE- AND ISOQUINOLINE GLUCOALKALOIDS

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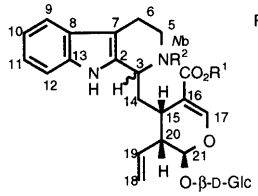
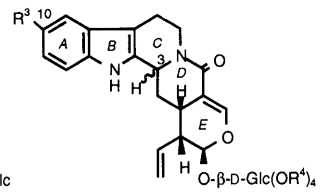
The highly shifted acetyl NMR signal of strictosamide tetraacetate, one of the most important reference molecules in the glucoindole alkaloid fields, is that on C2' of the glucose moiety. The signal that behaves similarly in methylisoalangiside tetraacetate was also confirmed to be that on C2'.

KEY WORDS strictosamide; methylisoalangiside; indole alkaloid; isoquinoline alkaloid; glucoside

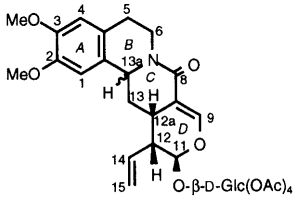
Strictosidine **1**,[1, 2] was first isolated from *Rhazya stricta* in 1968, and is now believed to be the universal intermediate in the biosyntheses of over 1400 monoterpenoid indole alkaloids.[3] Zenk and Kutchan[2a] purified strictosidine synthase, an enzyme that catalyzes strictosidine formation from tryptamine and secologanin. They also succeeded in the cloning of this enzyme, and this series of accomplishments was recognized as a highlight in the research field of biosynthesis of secondary metabolites. The history of the early stage of structural work on strictosamide and vincoside (3-*epi*-strictosidine) **2** was confusing.[3] These two amorphous solids differ only in stereochemistry at the diastereotopic C-3 position and behave quite similarly, as expected from the fact that both are glucosides with closely related structures. In the late 1960s and early 1970s FT-NMR instruments were not in general use and spectral measurements did not give convincing data that differentiated one from the other. G. N. Smith *et al.* finally determined the C3 configuration of **1** to be *S* through chemical correlation with dihydroantirhine, whose absolute configuration was known.[4] During this research they found that strictosamide tetraacetate **3** showed one highly shifted acetyl NMR signal at δ 1.22 ppm[4, 5] while C-3 epimer, vincoside lactam tetraacetate **4**, did not show such a phenomenon. This characteristic finding gave the first firm base for differentiating strictosidine from vincoside, and eventually the long controversy on stereostructual assignment and biogenetical intermediary came to an end. Since then, many structures of glycosidic indole alkaloids have been clarified through chemical correlation with derivatives of strictosidine and vincoside, to which the above key observations made enormous contributions. Similar observations have been reported in the isoquinoline glucoside field; Höfle *et al.*,[6] reported that one acetyl group in methylisoalangiside



1 R^1=Me, R^2=H, H3-α (*S*)
2 R^1=Me, R^2=H, H3-β (*R*)
5 R^1=H, R^2=H, H3-α
6 R^1=Me, R^2=Me, H3-α



3 R^3=H, R^4=Ac, H3-α
4 R^3=H, R^4=Ac, H3-β
7 R^3=H, R^4=H, H3-α
8 R^3=OAc, R^4=Ac, H3-α



9 H13a-α
10 H13a-β

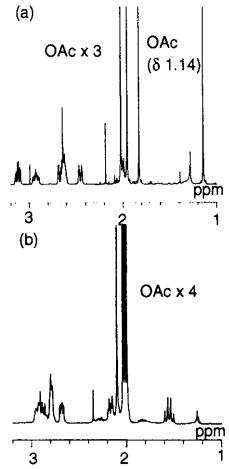


Fig. 1. Partial ^{1}H-NMR Spectra
(a) strictosamide tetraacetate **3** in CD_3OD and (b) vincoside lactam tetraacetate **4** in $CDCl_3$.

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tetraacetate **9** was observed in an anomalously high field at δ 1.57 ppm, while all acetyl signals of methylalangiside tetraacetate were observed at the normal position. As explained above, the presence of a highly shifted acetyl signal in glucosidic indole and isoquinoline alkaloids plays an important role, but no study has been carried out to specify which particular acetyl group on the sugar part of strictosamide tetraacetate **3** or methylisoalangiside tetraacetate **9** shows this abnormality. In this paper, we describe a detailed chemical and spectroscopic study that allowed us to conclude that the C-2' acetyl methyl is the active group.

First, strictosamide tetraacetate **3** was prepared. We used strictosidinic acid **5**[7, 8] from *Hunteria zeylanica* as the starting material. Diazomethane methylation gave strictosidine **1** and *N*b-methyl strictosidine **6**,[9] in 43.6% and 16.8% yield, respectively. Treatment of **1** with 15% aqueous sodium carbonate gave strictosamide **7**. Subsequent acetylation with acetic anhydride-pyridine yielded the tetraacetate derivative **3**.[10] In the ^{1}H-NMR spectrum of the CD_3OD solution, four acetyl signals were observed at δ 1.14, 1.83, 1.95, and 2.02 ppm. The HMBC spectrum revealed that the acetyl methyl proton at δ 1.14 coupled with a carbonyl carbon at δ 170.42, which, in turn, coupled with a proton signal at δ 4.64. The proton at δ 4.64 was proved to be H2' in the glucose part because a vicinal HH coupling was observed between this proton and the acetal proton (Hl') at δ 4.90 with J=8.1 Hz. The assignment of H1' acetal proton was evident from the observed one-bond coupling with acetal carbon (C1') at δ 95.89 on the HSQC spectrum. Tracing back this correlation, we found that the δ 1.14 acetyl methyl signal can be ascribed to that on C2' acetyl group of strictosamide tetraacetate **3**.

An acetyl signal at the 2' position in the pentaacetyl derivative of 10-hydroxystrictosamide **8**[11] was found to resonate at δ 1.24 in the ^{1}H-NMR spectrum, as in the case of **3**. This additional new finding indicates that the anomalously high field chemical shift of the 2' acetyl group is generally applicable to the C-3 stereochemical assignment of the strictosamide/vincoside lactam series.

Höfle *et al.*[6] reported that one acetyl group in methylisoalangiside tetraacetate **9** was also observed in an anomalously high field at δ 1.57 ppm. We synthesized **9** and the C13a epimer **10** according to the procedure reported in the literature,[6] *i.e.*, through condensation of dopamine hydrochloride and secologanin, lactamization under basic conditions, methylation of resulting phenols with diazomethane, and finally acetylation with acetic anhydride-pyridine. The stereochemisty at C13a in **9**[12] and **10** was shown by the CD spectra[13] that displayed the opposite Cotton effects in the longest wavelength absorption at around 280 nm.[14]

In the same way as in the case of strictosamide tetraacetate **3**, the HMBC and other measurements revealed that the acetyl methyl signal appearing at the highly shifted position was ascribable to that at the 2' position in **9**.

Next, we studied the conformation of the molecules using differential NOE. Irradiation at the 2' acetyl group of **3** caused the enhancement of H-17 (2.8%) and H-15 (1.8%) signals, and similarly, irradiation at the 2' acetyl group of **9** caused enhancement of H-9 (3.7%) and H-12a (1.3%) signals. These findings allowed us to deduce the relative disposition of the D/E ring of the skeletal part of **3** and **8**, the C/D ring of **9**, and the sugar part. The conformation of the C/D ring part of **3** and **8**, and the B/C ring part of **9** can be deduced from NMR spectral analysis. The structure of the whole molecule is constructed as shown in Figure 2. It is obvious that the 2' acetyl methyl group is located in the shielding area formed by the indole nucleus of **3** and **8** or the benzene ring of **9**.

The depicted conformation of the E-ring of strictosamide tetraacetate **3**, in which glucosyloxy and vinyl groups comprise the axial positions, is in good accord with Hutchinson's view obtained through the investigation of ^{13}C-NMR of **3**[15] and X-ray analysis of a vincoside derivative.[16]

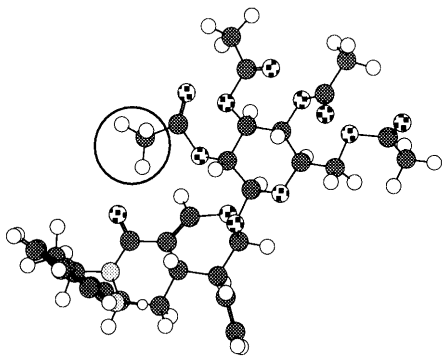

Fig. 2. Constructed Molecular Structure of Strictosamide Tetraacetate **3** on the Basis of NMR Spectral Data

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9) The study on compound **6** will be published in a forthcoming paper.
10) Spectral data of **3**; FAB-MS (NBA) *m/z*: 667 (MH^+, 67%), 666 (43), 331 (15), 307 (28), 289 (17), 169 (58), 154 (100), 136 (66). High-resolution FAB-MS *m/z*: 667.2523 (calcd for $C_{34}H_{39}O_{12}N_2$: 667.2503). ^{1}H-NMR (500 MHz, CD_3OD); δ: 7.36 (d, *J*=7.1, 9-H), 7.35 (s, 17-H), 7.34 (d, *J*=8.0, 0.8, 12-H), 7.09 (td, *J*=8.0, 1.2, 11-H), 6.98 (td, *J*=7.1, 0.8, 10-H), 5.63 (dt, *J*=17.4, 10.0, 19-H), 5.38 (dd, *J*=17.4, 1.7, 18-H), 5.32 (dd, *J*=10.0, 1.7, 18-H), 5.27 (d, *J*=1.5, 21-H), 5.06 (br-d, *J*=3.9, 3-H), 4.91 (dd, *J*=12.2, 6.1, 5b-H), 3.12 (td, *J*=12.2, 4.9, 5a-H), 2.93 (dddd, *J*=14.9, 12.0, 6.1, 2.4, 6-H), 2.67 (dd, *J*=15.4, 4.9, 6-H), 2.64-2.62 (2H, m, 20-H and 15-H), 2.46 (ddd, *J*=14.2, 3.9, 2.2, 14-H), 1.99 (td, *J*=14.2, 5.9, 14-H), 5.15 (dd, *J*=9.5, 9.5, 3'-H), 4.93 (dd, *J*=10.0, 9.5, 4'-H), 4.90 (d, *J*=8.1, 1'-H), 4.64 (dd, *J*=9.8, 8.1, 2'-H), 4.25 (dd, *J*=12.4, 4.4, 6'-H), 4.10 (dd, *J*=12.5, 2.2, 6'-H), 3.86 (ddd, *J*=10.0, 4.4, 2.4, 5'-H), 2.02 (3H, s, 6'-OAc), 1.95 (3H, s, 4'-OAc), 1.83 (3H, s, 3'-OAc), 1.14 (3H, s, 2'-OAc). ^{13}C-NMR (125 MHz, CD_3OD); δ: 172.27 (6'-C=O), 171.42 (3'-C=O), 171.20 (4'-C=O), 170.42 (2'-C=O), 166.80 (C-21), 148.23 (C-17), 138.00 (C-13), 133.55 (C-19), 128.72 (C-8), 122.51 (C-11), 121.03 (C-18), 112.52 (C-12), 110.21 (C-16), 110.14 (C-7), 96.47 (C-21), 95.89 (C-1'), 73.58 (C-3'), 73.23 (C-5'), 71.77 (C-2'), 69.70 (C-4'), 62.86 (C-6'), 55.24 (C-3), 45.11 (C-5), 43.99 (C-20), 26.93 (C-14), 25.24 (C-15), 22.05 (C-6), 20.56 (6'-OAc), 20.49 (4'-OAc), 20.37 (3'-OAc), 19.40 (2'-OAc).
11) Detailed experimental data will be reported elsewhere.
12) Selected spectral data of **9**; FAB-MS *m/z*: 688 (MH^+, 37%), 460 (5), 307 (33), 154 (100), 136 (64). High-resolution FAB-MS *m/z*: 688.2628 (calcd for $C_{34}H_{42}O_{14}N$: 688.2605). ^{1}H-NMR (400 MHz, $CDCl_3$); δ: 4.84 (d, *J*=8.0, 1'-H), 2.08 (3H, 6'-OAc), 2.01 (3H, 4'-OAc), 1.95 (3H, 3'-OAc), 1.57 (3H, 2'-OAc). ^{13}C-NMR (100 MHz, $CDCl_3$); δ 170.57 (6'-C=O), 169.99 (3'-C=O), 169.38 (4'-C=O), 168.98 (2'-C=O), 56.28 (2-OMe), 55.94 (3-OMe), 20.70 (6'-OAc), 20.53 (4'-OAc), 20.53 (3'-OAc), 19.73 (2'-OAc).
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14) CD spectra of **9** and **10**; compound **9**: λ_{ext} (EtOH) nm (Δε) 204.2 (0), 208.2 (+2.95), 213.0 (0), 224.2 (-7.28), 244.8 (-1.62), 250.6 (-2.27), 270.4 (0), 283.2 (+0.54), 307.6 (0); compound **10**: λ_{ext} (EtOH) nm (Δε) 204.2 (+31.6), 218.0 (0), 234.2 (-13.9), 298.2 (0).
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