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PHYTOCHEMISTRY

β-Carboline glucoalkaloids from *Strychnos mellodora*

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Abstract

Two new N_b-methylated β-carbolinium glucoalkaloids, 3,4,5,6-tetradehydropalicoside and 3,4,5,6-tetradehydrodolichantoside, together with the known β-carboline compounds desoxycordifoline (β-carboline 3-carboxylate glucoalkaloid) and melinonine F (N_b-methylated harmanium cation), were isolated from *Strychnos mellodora* stembark. The structures of the compounds were elucidated on the basis of spectroscopic studies.

Keywords: *Strychnos mellodora*; Loganiaceae; β-Carboline; Glucoalkaloids; Anhydronium alkaloids; 3,4,5,6-Tetradehydropalicoside; 3,4,5,6-Tetradehydrodolichantoside; Desoxycordifoline; Melinonine F; 2D-NMR

1. Introduction

Strychnos mellodora S. Moore is an East-African endemic species. It is a tree, growing up to 35 m in height and distributed in the mountain rainforests (800–1200m) of Tanzania, Zimbabwe and Mozambique (Leeuwenberg, 1969). The only phytochemical research on this plant was part of a large screening of 69 *Strychnos* African species carried out in 1971 by Bisset and Phillipson, who showed the presence of tertiary alkaloids (Bisset & Phillipson, 1971). A first investigation on this plant in our laboratory has led to the isolation and identification of three indolic glucoalkaloids: dolichantoside (**6**), palicoside (**5**) and strictosidine (**7**) (Tits et al., 1996). In continuation of our studies on the alkaloidal composition of *S. mellodora* stembark, we isolated three N_b-methylated β-carbolinium compounds (**1**, **2** and **4**) and a β-carboline glucoalkaloid (**3**), which were all characterized by a blue fluorescence under UV light. This paper deals with the isolation and structural elucidation of the new compounds, 3,4,5,6-tetradehydropalicoside (**1**) and 3,4,5,6-tetradehydrodolichantoside (**2**) (UV, IR, MS and NMR), as well as with the isolation and the identification of the known compounds, desoxycordifoline (**3**) (UV, MS and NMR) and melinonine F (**4**) (TLC, HPLC, UV and MS). We finally present an HPLC method for the identification of the main alkaloids of *S. mellodora* stembark.

2. Results and discussion

An EtOH extract of the dried powdered stembark of *S. mellodora* was separated by MPLC using a gradient of MeOH in Me_2CO. The initial fractions contained dolichantoside (**6**) and compound **3**, while the latter afforded a mixture of palicoside (**5**) and compounds **1**, **2**, **4**. Subsequent chromatography was needed to separate these polar, fragile products.

Compound **1**, positive ES-mass spectrum m/z 527 $[M]^+$ corresponding to the molecular formula $C_{27}H_{30}N_2O_9$, showed an intense blue fluorescence under UV light. The wavelengths of its λ_{max} in acidic or neutral solutions suggested a β-carbolinium chromophore. The bathochromic shift in alkaline solution

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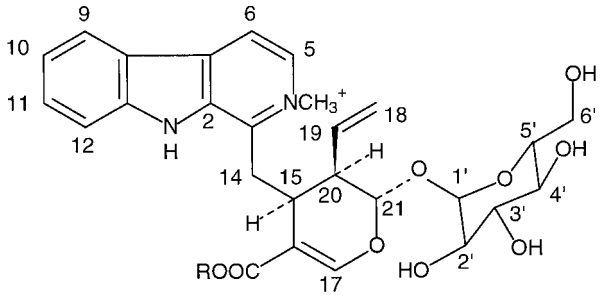

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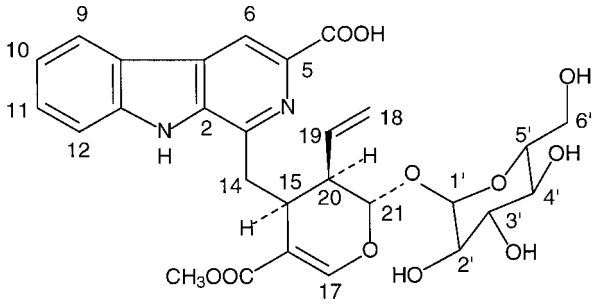

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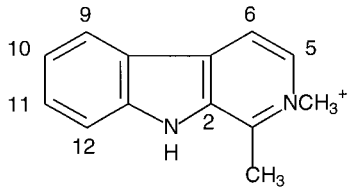

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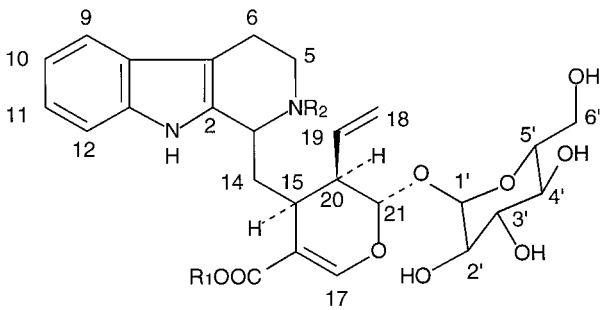

5: R^1=H, R^2=CH_3
6: R^1=CH_3, R^2=CH_3
7: R^1=CH_3, R^2=H

corresponded to the zwitterionic form (pH-dependent) anhydronium base (Gribble, 1988). The presence of a β-carboline derivative, as suggested by the UV spectrum, was confirmed by characteristic mass fragments at m/z 155, 167, 182 and 196 (Caprasse, Coune, & Angenot, 1983). The peak at m/z 363 indicated the loss of a sugar moiety. Cleavage with β-glucosidase established that the sugar moiety was β-D-glucose.

A more detailed understanding of the molecular structure of **1** was gained from its NMR spectra (Tables 1 and 2). In the 400 MHz ^{1}H NMR spectrum, the presence of a β-carbolinium structure was confirmed by the presence of six aromatic protons, four of which corresponded to an unsubstituted indole moiety and two more deshielded doublets whose chemicals shifts were in accordance with a pyridinium ring. The NMR data showed the presence of a quaternary N_b-methyl group (^{1}H: 4.35 (s), ^{13}C: 45 ppm). The deshielded values for the methyl protons are consistent with those of melinonine F and normelinonine F (^{1}H: 4.20, ^{13}C: 43.1 and ^{1}H: 4.55, ^{13}C: 46.3 ppm, respectively) (Caprasse et al., 1983] or other similar molecules, like chrysotricine (^{1}H: 4.42, ^{13}C: 45.2 ppm) (Peng, Feng, Zheng, & Liang, 1997). Several resonances suggested the presence of a seco-iridoid moiety similar to secologanin (Stevens, 1994). There was a singlet at δ 7.44 corresponding to the olefinic H_{17} and a doublet at δ 5.95 for the hemiacetalic H_{21}. The vinyl group was identified by doublets at δ 5.31 (J=10.8 Hz) and δ 5.23 (J=17.9 Hz) corresponding to the methylenic protons of C_{18}, which are respectively *cis* and *trans* coupled and by the unique hydrogen H_{19} (doublet of doublets at 5.97 ppm). A doublet at δ 4.78 with a coupling constant of 7.9 Hz, indicating β-configuration of the linkage, was consistent with the anomeric proton of a β-glucose moiety (Agrawal, 1992). This was supported by a complex set of resonances in the range 3.15–3.92 ppm. Moreover, the ^{13}C-NMR spectrum showed the sugar to be in the β-D-glucopyranose form as in dolichantoside, palicoside and strictosidine (Stevens, 1994; Tits et al., 1996)]. The ^{1}H-NMR spectrum revealed the absence of an *O*-methyl group. Two bands in the IR spectrum at ν_{max} 1635 and 1385 cm^{-1} suggested the presence of a β-alkoxyacrylate function. This was demonstrated by the fact that methylation of **1** with diazomethane gave the methyl ester **2**. Furthermore, the results of the COSY spectrum showed that in the aliphatic region a larger structural fragment could be assembled. The H_{18a}–H_{18b}–H_{19} vinylic protons afforded a convenient entry point in this system. The connectivities provided the means of assembling the multispin substructure related with the hydrogens bonded to C_{18}–C_{19}–C_{20}– (C_{15}–C_{14})–C_{21} which further confirmed the vincosan-type skeleton (no N_4C_{17} or N_4C_{21} bond). Thus, the structure of **1** was established as 3,4,5,6-tetradehydropalicoside. 2D NMR experiments (^{1}H–^{1}H COSY and HMQC) were used to establish the ^{1}H and ^{13}C assignments, which confirmed the proposed structure.

Table 1
^{1}H NMR spectral data of compounds **1**, **2**, **3** and **7** in CD_3OD (δ in ppm and J in Hz)

H	**1**	**2**	**3**	**7**[a]
3	–	–	–	3.96 dd
5a	8.34 d	8.28 d (6.3)	–	3.29 ddd
5b	–	–	–	2.93 ddd
6a	8.34 d	8.36 d (6.3)	8.69 (1H) s	2.80 ddd
6b	–	–	–	2.69 ddd
9	8.24 d (8.0)	8.31 d (8.0)	8.19 d (7.8)	7.35 dd
10	7.33 t (8.1)	7.36 t (8.0, 7.3)	7.28 t (7.8, 7.3)	6.94 ddd
11	7.66	7.71 t (7.3, 8.3)	7.56 t (7.3, 7.7)	7.01 ddd
12	7.66	7.75 d (8.3)	7.59 d (7.7)	7.23 dd
14a	3.6 m	3.75 m	3.43 m	2.04 ddd
14b	3.5 m	3.65 m	3.27 m	1.96 ddd
15	3.45 m	3.50 m (3.4)	3.67 m	3.03 ddd (4.6)
17	7.44 (1H) s	7.51 (1H) s	7.59 (1H) s	7.67 (1H) s
18_{trans}	5.23 d (17.9)	5.46 d (17.1)	4.67 d (17.3)	5.30 dd (17.3)
18_{cis}	5.31 d (10.8)	5.35 d (10.2)	4.93 d (10.7)	5.20 dd (10.6)
19	5.97 dd (10.8, 17.9)	6.04 ddd (10.2, 17.1, 8.0)	5.67 ddd (10.7, 17.3, 7.2)	5.84 ddd (10.6, 17.3, 7.6)
20	2.72 d (8.9)	2.83 ddd (9.4, 8.0, 3.4)	2.62 dd (7.3, 7.2)	2.67 ddd (9.0, 7.6, 4.6)
21	5.95 d (8.9)	6.15 d (9.4)	5.86 d (7.3)	5.82 d (9.0)
N^+–CH_3	4.35 (3H) s	4.44 (3H) s	–	–
COOH	n. d. in CD_3OD	–	11.5 s[b]	–
O–CH_3	–	2.90 (3H) s	3.54 (3H) s	3.74 (3H) s
1′	4.78 d (7.9)	4.91 d (7.9)	4.78 d (7.8)	4.78 d (7.9)
2′	3.15 d (7.9)	3.27 d (7.9)	3.19 d (7.8)	3.23 dd (7.9, 8.8)
3′	3.35 m	3.30 m	3.39 m	3.40 dd (8.8, 8.8)
4′	3.22 m	3.32 m	3.22 m	3.24 dd (8.8, 8.8)
5′	3.35 m	3.45 m	3.39 m	3.35 ddd (2.3, 6.7, 8.8)
6′a	3.92 d (10.8)	4.01 d (11.9)	3.99 d (10.7)	3.94 dd (11.8, 2.3)
6′b	3.60 d (10.8)	3.76 d (11.9)	3.67 d (10.7)	3.64 dd (11.8, 6.7)

[a] From Stevens (1994).
[b] Observed in DMSO-d_6.

Compound **2** was separated from **1** by prep. TLC on silicagel using MeOH–NH_4NO_3, 1 M–NH_4OH, 2 M (7:2:1) as mobile phase. This product emitted the same blue fluorescence under UV light and pH-dependent UV spectrum as **1**. The m/z 182 and 196 fragments in the ES^+ mass spectrum as well as the typical non-substituted indole nucleus and 3-substituted pyridinium ring protons in the down-field region of the ^{1}H NMR spectrum (Table 1) afforded the evidence for the presence of a β-carbolinium structure similar to **1**. Again we noted the presence of a deshielded singlet signal for a quaternary N_b-methyl group (^{1}H: 4.44, ^{13}C: 44.5 ppm). The m/z 541 $[M]^+$ peak from ES^+ mass spectrometry led to the molecular formula $C_{28}H_{32}N_2O_9$. Thus, **2** was heavier than **1** by 14 mμ, which could be consistent with the methylation of the carboxylic function of **1**. Indeed, the ^{1}H and ^{13}C NMR spectral data of compound **2** (Tables 1 and 2) were analogous to those of compound **1**, except for the signal at ^{1}H: 2.90 (^{13}C: 51 ppm), attributable to a carbomethoxy group. This chemical shift reflected a shielding of the methyl protons, like in other indolic alkaloids as 10-hydroxypericyclivine and derivatives (Pinchon et al., 1990). The examination of the molecular stereomodels in the case of N_b-methyl-β-carbolinium and N_b-methyltetrahydro-β-carboline alkaloids can partially explain the difference in shifts between the carbomethoxy protons in **2** and dolichantoside (**6**) (^{1}H: 3.70, ^{13}C: 51.9) (Coune & Angenot, 1978). The steric dimensions of the quaternary N_b-methyl group as well as the planarity of the β-carbolinium nucleus influence the position of the methyl ester in such a way that preponderance of its position in the shielding zone of the aromatic ring current from the β-carbolinium moiety becomes plausible. Furthermore, reaction of diazomethane with **1** provided the methylester **2**, which allowed us to assign the structure of **2** as 3,4,5,6-tetradehydrodolichantoside. This is believed to be the first report on the isolation of N_b-methylated β-carbolinium glucoalkaloids from nature.

The stereochemistry remained to be considered. The similarities between the chemical shifts of C_{15}, C_{20} and C_{21} in the new compounds **1** and **2** and in strictosidine (**7**) (Table 2) suggested the same configuration for these carbons in the two alkaloids. The weak deshielding effect observed for C_{15} and C_{20} can be attributed to the quaternary character of the N_b-methyl group. Moreover the coupling constants in **2** between H_{15},

Table 2
^{13}C NMR spectral data of compounds **1, 2, 3** and **7** in CD_3OD (δ in ppm). n.d. = not detected

C	1	2	3[a]	7[b]
2			135.6	137.7 or 136.3
3			142.9	51.7
5	135.9	133.5	135.6	43.2
6	117.1	116.0	114.2	22.4
7			128.4	108.4
8			121.7	128.5
9	124.3	124.0	121.4	118.5
10	123.1	121.8	119.9	119.6
11	132.8	132.0	128.4	121.9
12	114.1	115.0	111.6	111.8
13			141.6	137.7 or 136.3
14	32.0	31.5	34.0	37.1
15	36.0	35.5	34.5	32.7
16			108.7	110.8
17	155.0	155.0	153.2	155.3
18	120.3	119.5	117.6	119.1
19	135.6	n.d.	133.8	136.1
20	45.0	45.0	44.4	43.0
21	97.2	96.5	96.1	97.6
22			171.3	170.1
N^+–CH_3	45.0	44.5	–	–
O–CH_3	–	51.0	50.6	52.2
COOH	–	–	168.4	–
1′	100.8	100.5	99.0	100.3
2′	75.1	74.5	73.2	74.6
3′	78.0	78.0	76.6	78.0
4′	72.2	71.3	70.4	71.7
5’	79.1	78.0	76.6	78.7
6′	63.0	62.9	61.8	62.9

[a] Quaternary carbons deduced from the APT spectrum.
[b] From Stevens (1994).

H_{20} and H_{21} are consistent with the α-configuration of H_{15} and H_{20}, and the β-configuration of H_{21} (Levesque & Jacquesy, 1983). Thus, the proposed relative configurations of C_{15}, C_{20} and C_{21} are those commonly accepted from the biogenetical hypothesis: 15-$\alpha(S)$, 20-$\alpha(R)$ and 21-$\beta(S)$.

In the same fraction, compound **4** (m/z 197 $[M]^+$), which displayed a β-carbolinium UV spectrum, was identified as melinonine F after comparison by TLC and diode-array HPLC with an authentic sample isolated in our laboratory from *Strychnos usambarensis* roots (Caprasse et al., 1983).

The MPLC pooled fractions containing compound **3** and dolichantoside (**6**) were submitted to an HSCCC process to yield pure products. The UV data of **3** in basic, neutral and acidic conditions presented a bathochromic shift in acidic solution and were consistent with a fully aromatic β-carboline structure. In the $^1H^1H$ COSY NMR spectrum, a system of four aromatic protons showed that the benzenoid ring of the β-carboline was unsubstituted and a singlet at δ 8.69 indicated a substitution of the 5-position of the pyridinic ring. All characteristic resonances of a seco-iridoid moiety were present in the NMR spectra. In addition, the ES^+ mass spectrum providing a m/z 571 $[M+H]^+$ peak, suggested the presence of desoxycordifoline, an alkaloid previously isolated from *Adina* species (Rubiaceae) (Merlini & Nasini, 1968; Brown & Warambwa, 1978). The comparison of UV, MS and ^1H NMR spectra with those from literature confirmed this hypothesis. The ^1H NMR data of **3** are listed in Table 1. The ^{13}C NMR data in Table 2 do not seem to be available in previous literature. Moreover, this is the first time that a tryptophan-derived glucoalkaloid has been found in a member of the family of Loganiaceae.

Finally, a HPLC method for the analysis of alkaloids in the crude EtOH extract of *S. mellodora* stembark (**1**–**7**) was developed (see chromatogram in Fig. 1). The quantification of **5** (0.41%), **6** (0.66%) and **7** (0.018%) in *S. mellodora* stembark was performed using the corresponding references as external standards (Brandt, 1996). Using dolichantoside as external standard, the amounts of **1**, **2**, **3** and **4**, calculated with reference to the dried drug, showed that these compounds represented ca. 0.9% of β-carboline alkaloids. Adding up the previous results, we observe that the total amount of glucoalkaloids in *S. mellodora* is particularly high (ca. 2%) and superior to levels of alkaloids usually found in plants.

The biological activity of both new anhydronium bases glucosides **1** and **2**, especially the cytotoxic and antiparasitic properties, is now subject for further investigation. We have to consider those new products as leads for the development of new drugs given the encouraging results already obtained with β-carboline compounds especially in the field of cancerology, neurology and psychopharmacology (Rollema, Booth, & Castagnoli, 1988; Funayama et al., 1996; Malgrange et al., 1996; Picada, da Silva, Erdtmann, Henriques, & Henriques, 1997).

We have isolated six glucoalkaloids from *S. mellodora*, including strictosidine, the recognized exclusive precursor of all monoterpenoid indole and quinoline alkaloids (Phililipson & Zenk, 1980; Massiot & Delaude, 1988). This species, with significant amounts of those alkaloids, is a very primitive one from a phylogenetical point of view. It is a potential source of glucoalkaloids useful for biotechnological experiments and studies concerning the biosynthetic pathway of those alkaloids (Stevens, 1994).

3. Experimental

3.1. Plant material

The stembark of *S. mellodora* S. Moore was collected in Chirinda Forest (Zimbabwe) by CD. Voucher

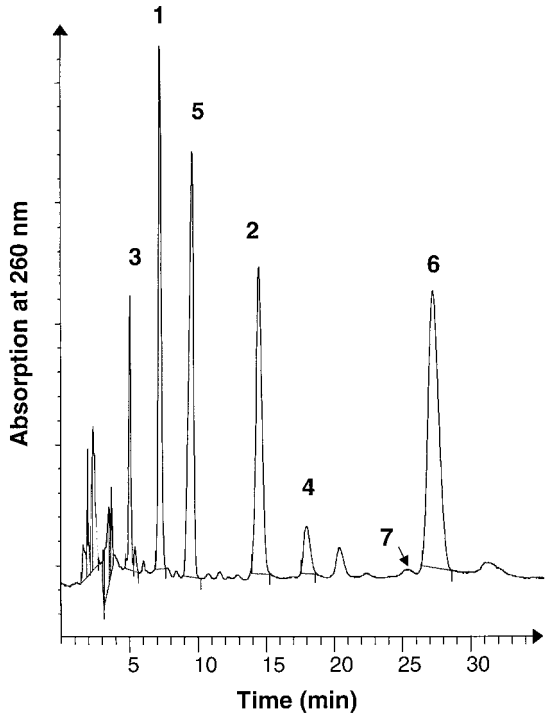

Fig. 1. HPLC profile of an EtOH/HOAc (99:1) extract of *Strychnos mellodora* stembark. See numbering in text.

specimens (L. Pauwels 7831) are kept in the Herbarium of the Botanical Garden of Belgium at Meise.

3.2. Extraction and isolation

Dried and powdered stembark (1 kg) was extracted with EtOH and EtOH–HOAc (99:1) at room temp. The combined extracts were conc. in vacuo to give 49 g residue. 4 g of this residue were fractionated by MPLC on a silica gel 60 using a Me_2CO/MeOH gradient. The Me_2CO/MeOH (99:1) fraction (266 mg) yielded **3** (10 mg) and **6** (109 mg) after purification by high speed counter current chromatography (HSCCC) in a multilayer-coil separator–extractor, fitted with a 2.6 mm i.d. coiled tubing and Me_2CO–*n*-BuOH–H_2O (1:8:10) as solvent. The upper organic phase was used as the stationary phase and the lower aq. phase as the mobile phase (descending mode). The (80:20) to (50:50) Me_2CO/MeOH fractions (637 mg) containing **1**, **2, 4** and **5** were firstly purified by HSCCC using $CHCl_3$–MeOH–H_2O (7:13:8) as solvent. The lower organic phase was used as stationary phase and the upper aq. phase as mobile phase (ascending mode). As **5** (70 mg) was obtained pure in this HSCCC system, compounds **1** (47 mg), **2** (67 mg) and **4** (13 mg) were finally separated by prep. TLC on silica gel (2 mm) in MeOH–NH_4NO_3, 1 M–NH_4OH, 2 M (7:2:1) (UV detection and elution by EtOH), followed by purification on Sephadex LH 20 eluting with MeOH.

3.3. 3,4,5,6-Tetradehydropalicoside (*1*)

Obtained as an amorphous brownish-yellow powder. UV (EtOH) λ_{max} nm ($\log \varepsilon$): 222 (3.97), 255 (3.06), 311 (2.85), 373 (2.31); (EtONa) λ_{max} nm ($\log \varepsilon$): 229 (3.88), 287 (3.29), 335 (2.70). IR (KBr) ν_{max} cm^{-1}: 3401, 2926, 1670 (sh), 1635, 1528, 1503, 1385, 1201, 1157, 1076, 944, 893, 825, 759. Positive ES–MS–MS *m/z*: 527 $[M]^+$, 364 [527-Glc]$^+$, 249 $[C_{17}H_{17}N_2]^+$, 221 $[C_{15}H_{13}N_2]^+$, 196 $[C_{13}H_{12}N_2]^+$, 182 [196-CH_2]$^+$, 167 [182-CH_3]$^+$, 155. ^1H and ^{13}C NMR data, recorded on a BRUKER 400 MHz spectrometer, are listed in Tables 1 and 2.

3.4. 3,4,5,6-Tetradehydrodolichantoside (*2*)

Obtained as an amorphous brownish-yellow powder. UV (EtOH) λ_{max} nm ($\log \varepsilon$): 209 (3.96), 254 (3.69), 311 (3.52), 375 (2.93); (EtONa) λ_{max} nm ($\log \varepsilon$): 213 (3.92), 288 (3.84), 336 (3.28), 438 (2.67). IR (KBr) ν_{max} cm^{-1}: 3401, 2925, 2854, 1690 (sh), 1635, 1527, 1503, 1384, 1245, 1196, 1157, 1076, 943, 825, 761. Positive ES–MS–MS *m/z*: 541 $[M]^+$, 379 [541-Glc]$^+$, 351, 347, 319, 221, 196, 182. ^1H and ^{13}C NMR data, recorded on a BRUKER DMX 600 MHz spectrometer, are listed in Tables 1 and 2.

3.5. Desoxycordifoline (*3*)

Obtained as a white powder. UV (EtOH) λ_{max} nm ($\log \varepsilon$): 239 (4.58), 268 (4.54), 336 (3.70), 349 (3.71); (EtONa) λ_{max} nm ($\log \varepsilon$): 240 (4.52), 260 (4.44), 268 (4.43), 285 (4.13), 337 (3.71), 352 (3.72); (EtOH + HCl) λ_{max} nm ($\log \varepsilon$): 220 (4.46), 240 (4.46), 278 (4.51), 376 (3.69). Positive ES–MS–MS *m/z*: 593 $[M+Na]^+$, 571 $[M+H]^+$, 409 $[M–Glc]^+$, 363, 359, 181, 167. ^1H and ^{13}C NMR data, recorded on a BRUKER DMX 600 MHz spectrometer, are presented in Tables 1 and 2.

3.6. Melinonine F (*4*)

UV, MS, ^1H NMR and ^{13}C NMR see lit (Caprasse et al., 1983).

3.7. Palicoside (*5*)

UV, MS, IR, ^1H NMR and ^{13}C NMR see lit (Morita et al., 1989).

3.8. Dolichantoside (*6*)

UV, MS, IR, ^1H NMR and ^{13}C NMR see lit (Coune & Angenot, 1978).

3.12. Strictosidine (7)

UV, MS, IR, ^{1}H NMR and ^{13}C NMR see lit (Smith, 1968; Stevens, 1994).

3.10. Enzymatic hydrolysis of glucoalkaloids

1 mg of substance and 5 mg of β-glucosidase from almonds (Merck) were allowed to stand at 30°C in a 0.1 M sodium phosphate buffer pH 6.3, for 24 h. After evaporation, the residue dissolved in 0.5 ml MeOH/H_2O (1:1) was analysed by TLC on silicagel using *n*-BuOH–Me_2CO–NaH_2PO_4 1.6% in water (4:5:1). Sugars were visualized with aniline phthalate reagent (Jork, Funk, Fischer, & Wimmer, 1990).

3.11. HPLC analysis

The stationary phase was a RP8 Lichrosorb SelectB column (5 μm, 250 × 4 mm) and heptanesulfonic acid (pH 3.7)/acetonitrile (75:25) at a flow rate of 1 ml/min was used as eluent. Alkaloids were detected at 260 nm by a diode-array detector. They appear in the following order (elution time in min): **3** (5.0), **1** (7.2), **5** (9.6), **2** (14.5), **4** (17.9), unidentified peak (20.4), **7** (25.3) and **6** (27.2).

3.12. Methylation of *1*

A MeOH solution of **1** (0.5 mg) to which an excess of CH_2N_2 in ether was added, was stirred for a few min after which the solvent was evaporated. A TLC system using MeOH–NH_4NO_3, 1 M–NH_4OH, 2 M (7:2:1) as mobile phase and UV detection showed that the product of methylation was **2**.

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