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ANTICANCER AND ANTIMICROBIAL GLYCOSIDES FROM *IPOMOEA BAHIENSIS**

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Key Word Index—*Ipomoea bahiensis*; Convolvulaceae; hydroxy fatty acid glycosides; structure; antimicrobial activity; tumour inhibition.

Abstract—Four new antimicrobial glycosides have been isolated from *Ipomoea bahiensis*. Spectroscopic properties and basic and acidic hydrolysis characterized them as derivatives of 11-hydroxyhexadecanoic and 11-hydroxytetradecanoic acid, glycosidically linked in the 11-position to a trisaccharide unit composed of glucose, rhamnose and fucose which is esterified by tiglic and 3-hydroxy-2-methylbutyric acid. One of these compounds revealed significant activity against Sarcoma 180 in mice.

INTRODUCTION

Numerous species of the genus *Ipomoea* are widely used in folk medicine all over the world especially as powerful cathartics [1]. Pharmacological studies reported antimicrobial [2, 3], analgesic [4], spasmogenic [5], spasmolytic [6, 7], hypotensive [8, 9], insecticidal [10], psychotomimetic [11] and anticancer [12, 13] effects. Chemical investigations indicate indole alkaloids [14] and resin glycosides [15] as the most common constituents in the Convolvulaceae.

In the course of our screening program of higher plants from north-eastern Brazil we found an exceptionally high number of *Ipomoea* species showing antimicrobial activity. Especially the methanolic extract of leaves of *I. bahiensis*† caused strong inhibition of growth of various representative microorganisms such as *Bacillus subtilis*, *Staphylococcus aureus*, *Neurospora crassa* and *Streptococcus faecalis*.

RESULTS AND DISCUSSION

In order to separate the active principle(s) of *I. bahiensis*, insoluble and inactive parts of the crude extract were separated by treatment with acetone and chloroform. The chloroform soluble part was fractionated by CC yielding 7.6% (dry wt) of a colourless resin which solidified on vacuum drying. Although TLC-analysis gave only one spot, HPLC showed at least three partially resolved peaks. Careful repetition of CC using a large excess of silica gel allowed us to isolate small amounts of four pure compounds, designated here as **1a**, **1b**, **2a** and **2b** in order of increasing polarity.

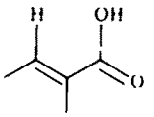
The glycosidic nature of all four compounds suggested by chromatographic behaviour was confirmed by IR and ^{1}H NMR spectroscopy. Nearly identical ^{1}H NMR spectra showed small olefinic multiplets, sugar protons and strong paraffin like aliphatic absorptions, but they did not allow us to establish any structural details. Therefore, we tried to obtain more information by chemical degradation. Alkaline hydrolysis of **1a** and **1b** liberated equimolar amounts of tiglic acid (**3**) [16] and 3-hydroxy-2-methylbutyric acid (**4**) [17], identified by ^{1}H NMR and IR spectroscopy. Compounds **2a** and **2b** yielded only **3**. No free sugar could be detected in the hydrolysate. Subsequent acidic hydrolysis allowed us to isolate 11-hydroxyhexadecanoic acid (**5a**) from both **1a** and **2a**, and 11-hydroxytetradecanoic acid (**5b**) from **1b** and **2b**. Compounds **5a** and **5b** were identified by melting points [18] and mass spectra of their methyl esters [19, 20]. In the aqueous layers of all acidic hydrolyses was found the same sugar mixture of glucose (**6**), fucose (**7**) and rhamnose (**8**) as evidenced by TLC and PC [21].

These results indicate that acids **3** and **4** must be present as esters, whereas the sugars **6**, **7** and **8** should be linked glycosidically. Since none of the glycosides showed acidic properties (IR, titration), the carboxyl group of **5a** and **5b** must also be esterified. Determination of saponification equivalent weight gave values of 311 and 302 for **1a** and **1b**, and 418 and 392 for **2a** and **2b**, respectively. If we assume monomeric structures, as suggested by chromatographic behaviour, we can calculate an M_r of 933 and 906 for **1a** and **1b** (three ester groups), and 836 and 784 for **2a** and **2b** (two ester groups).

In order to confirm these preliminary results and to establish the exact composition of the four glycosides, mass spectrometric determination of their M_rs was attempted. Whereas field desorption techniques did not result in satisfactory spectra, all four compounds produced strong and unambiguous $[M + H]^+$ and $[M + Na]^+$ peaks by the Fast Atom Bombardment (FAB) ionization [22] indicating molecular mass values of 890, 862, 790 and

*Part of this work was presented at the VIIth and VIIIth Simpósios de Plantas Medicinais do Brasil, Belo Horizonte, Sept. 1982 and Manaus, Sept. 1984, respectively.

†Herbarium DA-UFPE No. 5118, identified by A. A. Chiappeta.



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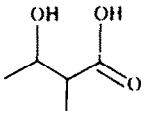

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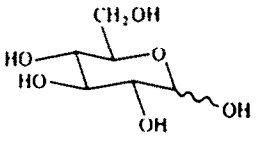
$Me(CH_2)_nCHOH(CH_2)_xCOOH$

5a $n = 4$
5b $n = 2$



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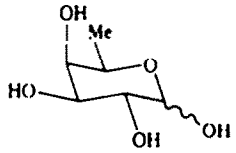

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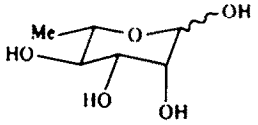

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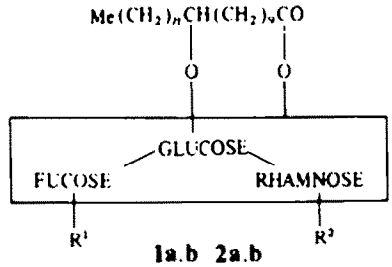
Fig. 1. Hydrolysis products of glycosides **1a**, **1b**, **2a** and **2b**.

762 for **1a**, **1b**, **2a** and **2b**, respectively. Based on these results we can deduce a general composition of one molecule of 11-hydroxy fatty acid **5a,b** linked glycosidically to a trisaccharide unit composed of glucose (**6**), fucose (**7**) and rhamnose (**8**) and esterified by one molecule of tiglic acid (**3**) in the case of **2a** and **2b**. Compounds **1a** and **1b** are esterified by an additional molecule of acid **4**. Further information about the sequence and branching of the trisaccharide unit is obtained from the FAB mass spectrum. The absence of both $[M+H-162]^+$ and $[M+H-146]^+$ peaks in the spectra of **1a** and **1b** suggests that there is no end unsubstituted sugar or desoxysugar. However, strong peaks corresponding to $[M+H-14-82]^+$ and $[M+H-146-100]^+$ indicate loss of two desoxysugars, one of which must be substituted by **3** and the other by **4**. On the other hand, the spectra of **2a** and **2b** show the expected $[M+H-146]^+$ peaks corresponding to the loss of an unsubstituted desoxysugar.

Only glycoside **1a** was available in sufficient quantity for the determination of a ^{13}C NMR spectrum. This spectrum showed the number, multiplicity and chemical shifts of peaks expected for the composition derived above, thus confirming the monomeric, triglycosidic nature of the compound and the presence of one molecule each of **3** and **4**. The 60 MHz ^1H NMR spectra of the four glycosides were now compared with the spectra of all hydrolysis products and with data from the literature. In all cases tiglic acid (**3**) was present, whereas hydroxy acid **4** was detected only in **1a** and **1b** by the presence of two doublets superimposed on the absorption of the fatty acid. No definite assignment of α- or β-configuration on the glycosidical links could be made because of the complexity of peaks. According to Wagner [15], sugars of the D-series (fucose and glucose) should have the β-configuration and L-rhamnose should be α-linked.

Finally, elemental analysis of all four glycosides were in perfect agreement with the composition and structural features found by hydrolysis and spectroscopic means. Summarizing all information obtained we can now propose the general structure as shown in Fig. 2.

Minimum inhibitory concentrations for antimicrobial activity were established by the streak method [23] for the unseparated mixture and for the pure glycosides, using 29



1a.b **2a.b**

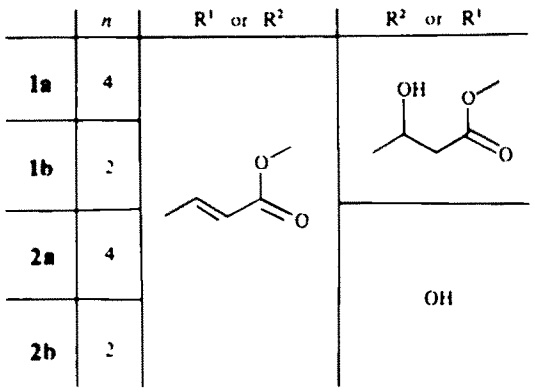


Fig. 2. General structure of glycosides **1a**, **1b**, **2a** and **2b**.

representative microorganisms. All four compounds and their mixture show medium activity against gram-positives, fungi are less affected and against gram-negatives no inhibition was observed below 500 μg/ml (Table 1). The only difference between the four compounds is a slight superiority of glycosides **1a** and **2a**, derived from 11-hydroxyhexadecanoic acid, against gram-positive microorganisms. All hydrolysis products, **3**, **4**, **5a** and **5b**, were inactive below 500 μg/ml.

All pure glycosides and their unseparated mixtures were submitted to antitumour tests in mice, using the transplantable tumors Sarcoma 180 and solid Ehrlich carcinoma [24]. After seven days of daily intraperitoneal application of the test compounds, animals were killed and tumour growth inhibition in relation to untreated control animals was determined. All compounds can be regarded inactive against solid Ehrlich carcinoma (inhibition lower than 50%); only **1a** and **1b** showed positive results against Sarcoma 180 (Table 2). Compound **1a**, even at a dose of 7.5 mg/kg causes considerable inhibition without any toxic side effects. Antitumour activity is much less pronounced in glycoside **1b**, whose only structural difference is a hydroxy fatty acid of two carbon atoms less. Compounds **2a** and **2b**, which lack esterification with acid **4**, as well as mixtures of two or more compounds can be considered inactive even at higher dose levels. These results indicate that antitumour activity is conditioned to the defined structure of the complete molecule. Small steric modification as in **1b** decreases the activity significantly and loss of one ester group renders **2a** and **2b** completely inactive.

Our observations represent the third demonstration of antitumour activity in *Ipomoea* species. *Ipomoea orizabensis* was reported to inhibit growth of Sarcoma 37 [12] and *I. leari* showed activity against Walker carcinosarcoma 256 [25]. From the latter was isolated the active compound named ipolearoside, a tetraglycoside derived from 3,11-dihydroxyhexadecanoic acid [13]. In view of these results it seems interesting to extend antitumour studies to glycosides or extracts of other Convolvulaceae. On the other hand, experiments with more representative tumour systems should be realized in order to evaluate a possible therapeutic application against human neoplasias. From the phytochemical point of view structures like **1a, b** and **2a, b** are typical of the Convolvulaceae [15], but in most cases polymeric resin glycosides are found and defined monomeric compounds are obtained only after basic hydrolysis. Glycosides present as monomers in the plant were described only in the case of *I. muricata* [26], *I. digitata* [5], *I. purpurea* [27] and *I. leari* [13]. Compared with these known glycosides, **1a, b** and **2a, b** are more complex, because they contain not only a hydroxy fatty acid and three different sugars but also short chain acids **3** and **4**. The unique structural features of Convolvulaceae glycosides and their multiple pharmacological properties should encourage more detailed studies in this field.

Table 1. Antimicrobial spectrum established by the streak method [23] of *Ipomoea* glycosides **1a, 1b, 2a, 2b** and their unseparated mixture

Tested microorganism	Minimum inhibitory concentration (μg/ml)				
	1a, b + 2a, b	**1a**	**1b**	**2a**	**2b**
B. subtilis 9	50–100	20–30	50–100	20–30	100–300
B. subtilis 27	50–100	20–30	50–100	20–30	100–300
B. anthracis	50–100	20–30	50–100	30–50	100–300
B. mycoides	50–100	20–30	50–100	50–100	100–300
S. aureus W	50–100	30–50	100–200	50–100	100–300
Sarc. lutea	50–100	20–30	30–50	30–50	100–300
E. coli S	> 500	> 500	> 500	> 500	> 500
S. typhosa	> 500	> 500	> 500	> 500	> 500
Ps. aeruginosa	> 500	> 500	> 500	> 500	> 500
Sh. paradysent.	> 500	> 500	> 500	> 500	> 500
Er. carotovora	> 500	> 500	> 500	> 500	> 500
St. faecalis	100–300	200–300	200–300	50–100	100–300
Br. suis	> 500	200–300	200–300	> 500	> 500
Br. abortus	> 500	200–300	200–300	> 500	> 500
Br. melitensis	> 500	200–300	200–300	300–500	> 500
M. tubercul. 607	100–300	200–300	50–100	100–300	100–300
M. smegmatis	100–300	200–300	300–500	100–300	100–300
M. phlei	100–300	30–50	200–300	100–300	100–300
C. albicans 50	100–300	200–300	> 500	100–300	100–300
C. krusei	> 500	> 500	> 500	> 500	> 500
C. tropicalis	> 500	> 500	> 500	> 500	> 500
N. catharralis	> 500	> 500	> 500	> 500	> 500
C. neoformans IHM	300–500	200–300	300–500	300–500	> 500
C. neoformans ENCB	300–500	200–300	200–300	300–500	> 500
N. asteroides	100–300	200–300	300–500	100–300	100–300
Pr. morganii	> 500	> 500	> 500	> 500	> 500
Pr. mirabilis	> 500	> 500	> 500	> 500	> 500
N. crassa	300–500	> 500	300–500	300–500	300–500
Asp. niger	> 500	> 500	300–500	> 500	> 500

Table 2. Tumour inhibition in mice of *Ipomoea* glycosides [24]

Substance	Dose (mg/kg)	Tumour	Inhibition (%)
1a	5	Sarcoma 180	15.8
	7.5	" "	67.5
	10	" "	70.1
	10	" "	71.9
	10	Solid Ehrlich Carc.	30.2
1b	10	Sarcoma 180	59.4
	15	" "	54.0
	20	" "	55.4
	10	Solid Ehrlich Carc.	30.9
1a + 1b	10	Sarcoma 180	51.0
	15	" "	50.0
	20	" "	23.0
	15	Solid Ehrlich Carc.	14.4
2a	10	Sarcoma 180	26.8
2b	10	Sarcoma 180	5.2
1a, b + 2a, b	10	Sarcoma 180	30.9
	20	" "	43.0
	15	Solid Ehrlich Car.	22.0

EXPERIMENTAL

Mps are uncorr. IR spectra were recorded in KBr pellets. ^{1}H NMR spectra are measured at 60 MHz in MeOH-d_4 with TMS as int. ref. and ^{13}C NMR spectra in the same solvent. EIMS were obtained at 70 eV and, FABMS by bombarding a glycerol soln of the glycosides with 4–6 keV Xenon atoms. TLC analysis of glycosides was effected on Polygram SIL G/UV plastic sheets (Macherey–Nagel) using $CHCl_3$–MeOH (17:3); spots were visualized by spraying with 50% H_2SO_4 and heating at 100°. All new compounds gave satisfactory C/H analyses (C ± 0.12; H ± 0.25). Saponification equivalent wt was determined by treatment with excess 0.01 N aq. NaOH and, after 24 hr at room temp., titration with 0.01 N aq. HCl (phenolphthalein).

Isolation of active fraction. Air dried, powdered leaves of *I. bahiensis* Willd. (200 g) were extracted at room temp. × 3 with 1 l. of MeOH. After filtration and evapn of solvent the brown resin (39.5 g) was treated with 200 ml of Me_2CO leaving 4.01 g of insoluble material without antimicrobial activity. The filtrate was concd to *ca* 50 ml and suspended in 150 ml of $CHCl_3$. Another insoluble and inactive fraction (6.80 g) was removed and the filtrate was concd *in vacuo*. The residue was fractionated on a column of 200 g of silica gel eluting with $CHCl_3$ containing increasing amounts (1–30%) of MeOH. The only active fractions, eluted with 5–10% of MeOH, yielded after evapn and vacuum drying 15.2 g (7.6% dry wt) of a colourless, amorphous powder, mp 90–92°, R_f 0.21.

Separation of glycosides **1a**, **1b**, **2a** *and* **2b**. The active fraction (200 mg) was fractionated by careful CC (200 g silica gel, $CHCl_3$–MeOH, 9:1) yielding 10–20 mg quantities of pure glycosides.

Compound **1a**. Mp 100–102°; R_f 0.24; IR ν_{max} cm^{-1}: 3420, 1722, 1655; ^{1}H NMR: δ6.95 (1H, *m*), 5.20–4.95 (4H, *m*), 4.45–3.40 (15H, *m*), 2.70–2.40 (3H, *m*), 1.83 (6H, *m*), 1.20 (3H, *d*, *J* = 7.0 Hz) and 1.16 (3H, *d*, *J* = 7.0 Hz) superimposed on 1.72–1.15 (30H, *m*), 0.90 (3H, *t*, *J* = 6.5 Hz). ^{13}C NMR: δ176.5 (*s*), 174.4 (*s*), 168.7 (*s*), 139.1 (*d*), 129.6 (*s*), 103.8 (*d*), 100.9 (*d*), 99.6 (*d*), 80.3 (*d*), 80.0 (*d*), 76.3 (*d*), 74.7 (*d*), 74.6 (*d*), 74.3 (*d*), 74.0 (*d*), 73.8 (*d*), 71.7 (*d*), 71.0 (*d*), 70.3 (*d*), 70.2 (*d*), 69.9 (*d*), 64.6 (*t*), 35.8 (*t*), 35.7 (*t*), 34.8 (*t*), 33.0 (*t*), 32.5 (*t*), 30.8 (*t*), 29.1 (*t*), 28.8 (*t*), 28.0 (*t*), 26.3 (*t*), 25.8 (*t*), 24.2 (*t*), 23.6 (*t*), 20.4 (*q*), 18.6 (*q*), 16.8 (*q*), 14.4 (*q*, 2 Me), 13.6 (*q*), 12.1 (*q*). FABMS: *m/z* 913 [M + Na]$^+$, 891 [M + H]$^+$. Sap. equiv. 311.

Compound **1b**. Mp 92–94°; R_f 0.23; IR ν_{max} cm^{-1}: 3420, 1722, 1655. ^{1}H NMR: δ6.95 (1H, *m*), 5.20–4.95 (4H, *m*), 4.45–3.40 (15H, *m*), 2.70–2.40 (3H, *m*), 1.83 (6H, *m*), 1.20 (3H, *d*, *J* = 7.0 Hz) and 1.16 (3H, *d*, *J* = 7.0 Hz) superimposed on 1.72–1.15 (26H, *m*), 0.90 (3H, *t*, *J* = 6.5 Hz). FABMS *m/z*: 885 [M + Na]$^+$, 863 [M + H]$^+$, 845 [M + H − H_2O]$^+$, 635 [M + H − 146 − 82]$^+$, 617 [M + H − 146 − 82]$^+$. Sap. equiv. 302.

Compound **2a**. Mp 128–130°; R_f 0.22; IR ν_{max} cm^{-1}: 3420, 1722, 1655. ^{1}H NMR: δ6.95 (1H, *m*), 5.20–4.95 (4H, *m*), 4.45–3.40 (14H, *m*), 2.70–2.40 (2H, *m*), 1.83 (6H, *m*), 1.72–1.15 (30H, *m*), 0.90 (3H, *t*, *J* = 6.5 Hz). FABMS *m/z* 813 [M + Na]$^+$, 791 [M + H]$^+$, 645 (M + H − 146]$^+$, 563 [M + H − 146 − 82]$^+$. Sap. equiv. 418.

Compound **2b**. Mp 134–136°; R_f 0.21; IR ν_{max} cm^{-1}: 3400, 1720, 1654. ^{1}H NMR: δ6.95 (1H, *m*), 5.20–4.95 (4H, *m*), 4.45–3.40 (14H, *m*), 2.70–2.40 (2H, *m*), 1.83 (6H, *m*), 1.72–1.15 (26H, *m*), 0.90 (3H, *t*, *J* = 6.5 Hz). FABMS *m/z*: 785 [M + Na]$^+$, 763 [M + H]$^+$. Sap. equiv. 392.

Alkaline hydrolysis. Quantities of glycosides **1a**, **1b**, **2a** and **2b** (100 mg) were stirred in a soln of 100 mg of NaOH in 2 ml of H_2O. After 4 hr at room temp. the soln was acidified with 4 N H_2SO_4 and extracted with CCl_4. NMR analysis of this soln identified tiglic acid (3) [16]. Further extraction of the aq. layer with EtOAc and evapn of the solvent gave in the case of **1a** and **1b** a colourless oil, identified by NMR and IR [17] as 3-hydroxy-2-methylbutyric acid (4). TLC analysis of the remaining aq. layer showed no free sugar. This acidic soln was refluxed for 8 hr and then extracted with $CHCl_3$. After removal of solvent and recrystallization from *n*-hexane, **1a** and **2a** yielded the same hydroxy acid **5a**, mp 63–65°, lit. [18] 62–64°. The EIMS of its Me ester, mp 42–43°, lit. [18] 41.5–42.5°, showed the typical [M − OMe]$^+$ peak at *m/z* 255 [20]. Similarly, **1b** and **2b** gave hydroxy acid **5b**, mp 47–49°, lit. [18] 49–50° and its Me ester, mp

31-32°, lit. [18] 30–31°; EIMS *m/z*: 227 [M − OMe]⁺ [19]. The aq. layer from these hydrolyses was neutralized with $KHCO_3$, evapd to dryness and the residue taken up in MeOH. TLC (silica gel, BuOH–H_2O–HOAc, 4:1:1) and PC [21] indicated in all cases the presence of glucose, fucose and rhamnose.

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