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GESHOIDIN: A BITTER PRINCIPLE OF *RHAMNUS PRINOIDES* AND OTHER CONSTITUENTS OF THE LEAVES

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ABSTRACT. The leaves and stems of *Rhamnus prinoides* are used in the preparation of domestic beverages *Tella* and *Tej*. Chemical investigations on the leaves resulted in the isolation and characterization of a bitter principle as the previously unknown ß-sorigenin-8-*O*-ß-D-glucoside for which the trivial name geshoidin is proposed. The structure of geshoidin was established by spectroscopic analysis of the natural product, the peracetate and permethylated derivatives. Other substances that have been identified include: ß-sorigenin, chrysophanol, physcion, musizin, emodin, rhamnocitrin, rhamnazin, quercetin and 3-*O*-methylquercetin.

INTRODUCTION

Rhamnus prinoides L'Herit, Amharic name: *Gesho*, family Rhamnaceae, is a plant which grows up to 6 meters. It is known to occur outside Ethiopia in Cameroon, Sudan, throughout East Africa to South Africa and Angola and also in Arabia [1]. It is cultivated in Ethiopia in view of its important application in the domestically brewed beverages of *Tella* and *Tej*. *Gesho* is an important commodity which is sold in almost every traditional market in Ethiopia. Although it is quite common to find *Gesho* cultivations throughout the country, it is worth mentioning that regions in Tigrai, around Kara Kori in North Shoa and Sebeta, just west of Addis Ababa, are important centers of production of *Gesho*. Travelers passing through Kara Kori will undoubtedly notice villagers sitting by the street side chopping *Gesho* branches and allowing them to dry all along the length of the highway passing through the town.

The leaves and stems of *Gesho* are indispensable ingredients in the making of the traditional fermented beverages *Tella* and *Tej*. *Tella* is a malt beverage, like beer, whose preparation begins with a starter called *Tinses* containing powdered leaves and stems of *Gesho* in water together with malted grains of barley, wheat, maize and sorghum or finger millet (*Bikil*). This mixture is allowed to ferment in a big round-bottomed earthenware called *Gan* or *Ensera* for about four days, after which are added small pieces of thin, toasted bread made from barley and wheat. The *Asharo* (dark roast of whole barley) is added on the fifth day. Further fermentation is allowed to go on for two to three days to give *Dif-dif*. The *Dif-dif* is either filtered to yield what is popularly known as *Filter-tella* or diluted with water and drank as a regular *Tella* [2, 3].

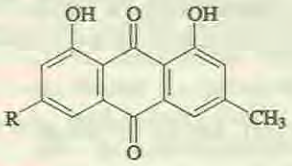
Although it is generally known that *Gesho* imparts the characteristic bitterness of these beverages, more precise understanding of the scientific role of this plant in this ancient brewing process is emerging only very slowly. The first scientific report on *R. prinoides* is

that of Salgues [4] who described the presence of inorganic cations, organic acids and the flavonoid derivative rhamnetin. He also claimed that the leaf extract was toxic to rabbits. This was followed by that of Coady in 1965 [5] who suggested that *Gesho* may have a role in the fermentation process. More concrete information on the microbiological role of *Gesho* came out in two publications [6, 7] where it is claimed that the plant regulates the microflora responsible for the fermentation process. These workers also indicated that the bitterness of the brew is directly related to the amount of *Gesho* added. Recently, efforts have been made overseas to develop technologies that allow the utilization of extracts from *Gesho* to hop beer [8]. Care is always taken to remove the fruits of the plant from the leaves and stems during the making of *Tella* and *Tej*. The fruits are, however, used for the treatment of ring worm infections. Prinoidin (**4**), a novel anthrone rhamnoside diacetate [9] and related compounds as well as other anthracene derivatives have been reported from the fruits of *R. prinoides* [10]. We have now investigated the chemical constituents of the leaves and fully characterized the previously unknown naphthalenic compound - ß-sorigenin-8-*O*-ß-D-glucoside (**6**), which is responsible for the bitter taste of the leaves. The name 'geshoidin' is proposed for this novel glucoside. Other substances that have been identified include: chrysophanol (**1**), physcion (**2**), emodin (**3**), prinoidin (**4**), musizin (**5**), ß-sorigenin (**8**), rhamnocitrin (**10**), rhamnazin (**11**), quercetin (**12**), and 3-*O*-methylquercetin (**13**).

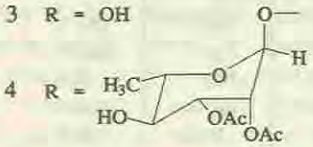
RESULTS AND DISCUSSION

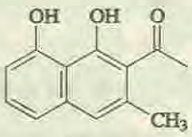
Methanol extraction of the leaves and removal of solvent led to 5.56% of residue. A portion of this was subjected to flash chromatography on silica gel. The less polar components from this column (petrol-EtOAc, 7:3) yielded a mixture, which was subjected to preparative layer chromatography to furnish the common anthraquinones chrysophanol (**1**), physcion (**2**), and the naphthalenic phenol, musizin (**5**). These compounds were identified by direct comparison with authentic samples which were available in our laboratories. Subsequent fraction from the above column also yielded a mixture of two substances, which was purified by passing through a column of Sephadex LH-20. This led to the isolation of the known common anthraquinone, emodin (**3**), and the flavonoid, rhamnocitrin (**10**). This fraction was found to contain trace amounts of flavonoids which were obtained in much higher yield when the plant was extracted using an alternate procedure (see below). The more polar eluates from the flash column, when let to stand for *ca* one hour, formed a copious amount of precipitate (see Experimental) which was found to contain the novel naphthalenic glucoside, geshoidin (**6**), as the major constituent. Geshoidin, mp 160-162°, $[\alpha]_D^{22}$ -114° (MeOH, c = 0.005) showed IR, ^1H and ^{13}C NMR properties which suggested that it was a naphthalenic lactone glycoside. The DEPT spectrum indicated the presence of two CH_2, nine CH and seven quaternary carbons and the absence of CH_3 group. This was consistent with the molecular formula $C_{18}H_{18}O_9$. Initially it was assumed that the compound may be the known ß-sorigenin-1-*O*-ß-glucoside (**7**) [11]. However, upon closer examination it was evident that the glucose ring was attached to the 8-OH group. Thus, the ^{13}C NMR data of geshoidin showed significant differences from those of **7** for C-4, C-5 and C-7, resulting from the different substitution pattern (See Table 1). Evidence for the presence of a non-glucosylated OH, which is chelated with the *peri* lactonic carbonyl, was obtained by observing a bathochromic shift with $AlCl_3$ in the UV spectrum. Hydrolysis of geshoidin gave a faint yellow aglycone (**8**). This aglycone displayed, in its ^1H NMR spectrum, four aromatic proton signals at δ 7.45, 7.36, 7.31 and 6.83, and the methylene protons on the lactone ring at δ 5.34. The spectroscopic data of this

compound were identical to those reported in the literature [11]. Examination of the aqueous hydrolysate with PC led to the detection of glucose.



1 R = H
2 R = OCH_3
3 R = OH





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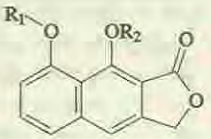
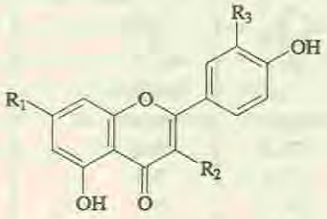


6 R_1 = Glucp R_2 = H
7 R_1 = H R_2 = Glucp
8 R_1 = H R_2 = H
9 R_1 = H R_2 = OCH_3



	R_1	R_2	R_3
10	OCH_3	OH	H
11	OCH_3	H	OCH_3
12	OH	OH	OH
13	OH	OCH_3	OH

Acetylation of geshoidin with pyridine-acetic anhydride yielded a peracetate. The infrared spectrum did not show any OH absorption band suggesting that the compound had been acetylated completely. ^{1}H NMR spectrum revealed the presence of four aliphatic acetate signals (δ 2.01-2.10), one aromatic acetate signal at δ 2.50, and four aromatic protons. Geshoidin was methylated by the method of Kuhn [12] to yield a permethylated derivative whose ^{1}H NMR spectrum showed four aromatic proton signals at δ 7.05, 7.49 and 7.52 (2H), an anomeric proton resonance at δ 5.13 (*d*, J = 7.5 Hz), and a lactone CH_2 at δ 5.34, and five methoxy signals. The ^{13}C NMR of this compound showed the aromatic methoxy carbon resonance at the low field value of δ 64.4 indicating that it is flanked between two quaternary carbons with substituents that force the methyl carbon into the deshielding zone of the ring. The ß-linkage of the sugar moiety was established from the ^{1}H NMR coupling constant (J = 7.5 Hz) of H-1' in the glucoside. Hydrolysis of the permethylated glucoside with methanolic HCl furnished sorigenin-1-*O*-methyl ether (**9**). The ^{1}H and ^{13}C NMR of this compound were

as expected and very similar to that of sorigenin (**8**) except for the appearance of a methoxy signal at δ 4.45 and δ 65.16 in the ^1H and ^{13}C NMR spectra, respectively. The down field location of the signal in the ^{13}C NMR spectrum is consistent with the location of a methoxy on a carbon that is *ortho* disubstituted - a further confirmation of the *O*-glucosylation of geshoidin at C-8.

Table 1. ^{13}C NMR spectral data of compounds **6**, **7** [11], and the peracetate of geshoidin.

Carbon No.	6[a]	7	6[b] peracetate
1	155.3[c]	155.9	147.3
2	114.3	113.8	120.3
3	139.5	142.7	140.3
4	111.0	119.4	118.4
4a	142.9	139.8	141.3
5	123.0	110.4	123.5
6	129.5	130.1	129.2
7	111.0	104.4	111.3
8	155.8[c]	156.9	155.2
8a	105.8	108.8	115.3
lactone-CH_2	67.9	68.2	68.2
lactone-CO	168.8	168.4	170.3[c]
1'	102.9	102.9	99.9
2'	73.4	73.3	72.1
3'	77.8	77.8	72.5
4'	69.9	69.7	68.2
5'	72.3	76.2	73.3
6'	60.8	60.7	62.0
$OCOCH_3$ (sugar Me)			20.5
$OCOCH_3$ (Ar-Me)			20.9
OC=O (Acetate)			170.1[c], 169.5
			169.4, 169.2
			167.6

[a]measured in $DMSOd_6$
[b]measured in $CDCl_3$
[c]interchangeable

A second extraction was undertaken in an effort to identify the flavonoids detected in the first extract discussed above. The powdered leaves were treated with 5% acetic acid, dried, defatted with petroleum ether, and extracted successively with chloroform and methanol. The methanol extract was found to be rich in flavonoids and other more polar constituents. The residue from the methanol extract was subjected to flash chromatography and the less polar eluate (chloroform solvent) was subjected to Sephadex LH-20 purification to yield rhamnazin (**11**), prinoidin (**4**) and sorigenin (**8**). Rhamnazin (**11**) was identified on the basis of its NMR spectrum (300 MHz) and by difnOe studies to confirm the locations of the methoxy groups at C-7 and C-3'. Prinoidin (**4**) was identified by direct comparison with an authentic specimen which was isolated previously from the fruits of *R. prinoides* [9]. Sorigenin (**8**) was identical to the substance obtained by hydrolysis of geshoidin (**6**), and also showed spectroscopic data

identical to those reported for the same substance in the literature [11]. It is not very clear to us whether sorigenin is a true natural product or an artefact formed by the breakdown of geshoidin. The next polar fraction from the flash chromatography was applied on a column of Sephadex LH-20 to yield rhamnocitrin (**10**) and a mixture of quercetin (**12**) and 3-*O*-methylquercetin (**13**). This mixture, which was not purified further, was analyzed by NMR and found to contain a (80/20) mixture of the two flavonoids, **12** and **13**, respectively. Later fractions from the flash column yielded geshoidin (**6**) but the quantities obtained in this case were far lower than the fractions found in the first extraction procedure (see above).

An organoleptic evaluation was made by locating five volunteers who were not aware of the research conducted in our laboratories. They independently confirmed that geshoidin possess bitter properties. It is interesting to note that geshoidin is bitter, despite the presence of a glucose moiety in the structure. In view of the fact that the plant is used as a bittering principle for domestically brewed beverages, and since this substance is present in considerable quantities in the leaves, it would be very worthwhile to conduct full biological evaluation of this substance. In this regard preliminary toxicological data was obtained [17] by assessing the toxicity of geshoidin to brine shrimp (*Artemia salina*). The effect of geshoidin on brine shrimp was examined in triplicate at seven different concentrations over a range of 64 folds (15 to 1000 μg/mL). No lethality was observed.

Naphthalene lactones are in fact common in the Rhamnaceae. As mentioned earlier, an isomer of geshoidin with the glucose unit on 1-OH, has been reported from *R. wightii* [11]. α-Sorimin has been reported from *Rhamnus pallasii* [13], *R. japonicus* [14, 15], and also in *Ventilago*, a genus in Rhamnaceae [16]. The co-occurrence of musizin (**5**) and geshoidin (**6**) in the leaves of the same plant undoubtedly has biosynthetic significance. It is most probable that musizin is a precursor of geshoidin.

EXPERIMENTAL

General. Mps: uncorr.; FT-IR KBr discs; ^{1}H NMR at 90 or 300 MHz; ^{13}C NMR at 22.4 and 75 MHz. ^{1}H and ^{13}C chemical shifts are referenced to the residual solvent signals. Analytical TLC: silica gel (Merck, Kiesel gel 60_{254}, 0.25 mm), flash CC: silica gel (Merck 9385 Kiesel gel 60, particle size 0040-0.063 m, impregnated with 5% aq. oxalic acid). Prep. TLC: silica gel (Merck 7748, Kiesel gel 60 $PF_{254+366}$, 1 mm).

Plant material. Rhamnus prinoides L'Herit leaves were purchased from the central market (*Merkato*) in Addis Ababa.

First extraction and isolation. Cold extraction of the powdered leaves (2.5 kg) in MeOH (5 L) for 72 h and removal of solvent *in vacuo* led to 139 g of residue. A portion of this residue (25 g) was subjected to flash chromatography on oxalic acid impregnated silica gel employing solvents of increasing polarity and 27 fractions were collected as follows: frs 1-2 (petrol/EtOAc 9:1), frs 3-10 (petrol/EtOAc 7:3), frs 11-16 ($CHCl_3$/EtOAc 2:1), frs 17-22 (EtOAc/MeOH 2:1), frs 23-26 (EtOAc/MeOH 1:1) and fr. 27 (MeOH).

The petrol-EtOAc (7:3) eluate gave mixtures of chrysophanol (**1**), physcion (**2**) and musizin (**5**). These were purified by prep. TLC using solvent system petrol-EtOAc (4:1). Frs 11-18 were combined, freed of solvent and applied on Sephadex LH-20 (solvent: $CHCl_3$-MeOH, 2:1) and the post chlorophyll eluates yielded emodin (**3**) and rhamnocitrin (**10**) together with a mixture of polar flavonoids whose trace amounts made their isolation and

identification difficult. Rhamnocitrin (**10**), pale yellow needles, mp 232-234° (Lit. [18] 221-223°); UV λ_{max} (EtOH) nm: 267, 327 (*sh*), 368; UV λ_{max} (EtOH + NaOAc) nm: 265, 327, 372; IR ν_{max} (KBr) cm^{-1}: 3261, 1663, 1609, 1505, 1233, 1164; ^1H NMR (300 MHz, CD_3OD): δ 3.88 (*s*, OMe), 6.29 (*d*, *J* = 2.2, H-5), 6.58 (*d*, *J* = 2.2, H-8), an A_2B_2 pattern at δ 6.90 (*d*, *J* = 8.82, H-2' and H-4'), 8.11 (*d*, *J* = 8.82, H-1' and H-5'). Frs 19-26, upon standing for 1 h, yielded copious precipitate, 11.4 g, with the largest quantity (7.2 g) obtained in one single fraction (fr. 20). One g of this precipitate was crystallized from MeOH to yield 378 mg of dull yellow crystals of geshoidin (**6**), mp 160-162°, $[\alpha]_D^{22}$ -114° (MeOH, c = 0.005), UV λ_{max} (MeOH) nm (log ϵ): 250.0 (4.63), 286.6 (4.02), 298.2 (4.17), 311.7 (4.2), 348.2 (4.37), 360.1 (4.4), UV λ_{max} (MeOH + $AlCl_3$) nm: 248.4, 289.9, 302.4, 315.5, 386.1, 400.9; IR ν_{max} (KBr) cm^{-1}: 3348, 1738, 1648, 1382, 1064; ^1H NMR (300 MHz, DMSO-d_6): δ 3.30-3.75 (6H, Glc), 5.06 (*d*, *J* = 7.8, H-1'Glc), 5.26 (lactone CH_2), 7.27 (*s*, H-4), 7.33 (*d*, *J* = 7.2, H-7), 7.45 (*t*, *J* = 7.3, H-6), 7.49 (*d*, *J* = 7.2, H-5), 10.45 (*s*, 1-OH); ^{13}C NMR (DMSO-d_6): See Table 1.

Second extraction and isolation. The powdered leaves (1.5 kg) were treated with 5% acetic acid and dried. They were defatted with petroleum ether and then successively extracted with $CHCl_3$ and MeOH. The MeOH extract (30 g) was flash chromatographed on 300 g of oxalic acid-impregnated Silica gel and 250 mL frs were collected. Frs 1-8 ($CHCl_3$), 9-12 ($CHCl_3$-EtOAc 4:1), 13-16 ($CHCl_3$-EtOAc 1:1), fr. 17 (EtOAc), frs 18-20 (EtOAc- MeOH 2:1) and fr. 21 (MeOH). Frs 2-6 was freed of solvent and applied on Sephadex LH-20 (solvent $CHCl_3$-MeOH 2:1). The fraction immediately following chlorophyll was a yellow solution which was evaporated to yield rhamnazin (**11**); mp 225-227°, (Lit. [11] 216-218°); IR ν_{max} (KBr) cm^{-1}: 3481, 3292, 1654, 1588, 1500, 1309, 1153, 1120; ^1H NMR (300 MHz, DMSO-d_6): δ 3.86 (*s*, OMe), 3.85 (*s*, OMe), 6.34 (*d*, *J* = 2.2 Hz, H-6), 6.77 (*d*, *J* = 2.2, H-8), 6.94 (*d*, *J* = 8.4, H-5), 7.74 (*dd*, *J* = 8.4, 2.1, H-6'), 7.78 (*d*, *J* = 2.1, H-2'), 12.48 (*s*, 5-OH). Frs 5-6 was also purified using Sephadex LH-20 and the post-chlorophyll fraction yielded a mixture of two pigments which were separated into a $CHCl_3$ soluble component (identified as prinoidin, **4** [9]) and a $CHCl_3$ insoluble part. The latter was re-applied on Sephadex LH-20 and eluted with methanol to yield 80 mg of sorigenin (**8**), mp 240-243° (Lit. [11] 245°); IR ν_{max} (KBr) cm^{-1}: 3413, 1713, 1654, 1380, 1099; UV λ_{max} (MeOH) nm: 250, 314, 364; ^1H NMR (300 MHz, DMSO-d_6): δ 5.34 (*s*, CH_2), 6.83 (*d*, *J* = 7.69, H-7), 7.31 (*s*, H-4), 7.36 (*br d*, *J* = 7.80, H5), 7.45 (*t*, *J* = 7.71, H-6), 10.45 (*s*, *peri*-OH).

Frs 7-10 was applied on Sephadex LH-20 and eluted with $CHCl_3$-MeOH (2:1) and the post-chlorophyll part yielded three fractions. The first contained more sorigenin, **8** (see above). The second fraction contained three components: prinoidin (**4**) [9], sorigenin (**8**) and a third component. The latter was obtained in a pure state from the third fraction of the Sephadex column and identified as rhamnetin (**10**) (See above).

Frs 11-15 was freed of solvent and applied on a Sephadex LH-20 column and eluted with $CHCl_3$-MeOH (2:1). The colored post chlorophyll portion was collected as one fraction. Removal of solvent furnished a precipitate which when treated with methanol left a residue which was characterized as sorigenin (**8**). The methanol solution was evaporated to give a mixture of quercetin (**12**) and 3-*O*-methylquercetin (**13**) Fraction 16 from the flash chromatography (see above) yielded a yellow precipitate when concentrated. A portion of this precipitate was purified on a Sephadex LH-20 column by elution with methanol to yield more of the mixture of quercetin (**12**) and 3-*O*-methylquercetin (**13**).

Frs 20-21 from flash chromatography was freed of solvent and applied on silica gel CC and eluted with EtOAc/MeOH/H_2O (77:13:10) and 20 fractions each of 50 mL were collected.

Frs 11-20 were combined, freed of solvent and re-applied on silica gel CC and eluted with C_6H_6/MeOH (4:1) to yield slightly impure geshoidin (**6**). A pure sample of geshoidin (28 mg) was obtained by purification with Sephadex LH-20 and elution with methanol.

Acetylation of geshoidin (6). One g of the crude precipitate obtained from flash chromatography was refluxed in 10 mL acetic anhydride and 2 drops of pyridine were added. The reaction mixture was stirred and refluxed for 3 h, cooled and added to 100 g of crushed ice and stirred for 30 min. The precipitate was filtered, washed with cold water to yield 460 mg of the peracetate, white solid, mp 282°, $[\alpha]_D^{22}$ -80° ($CDCl_3$ c = 0.005), UV λ_{max} ($CHCl_3$) nm: 215, 245, 299, 311, 349; IR ν_{max} (KBr) cm^{-1}: 3348, 1737, 1648, 1382, 1064; ^1H NMR (300 MHz, $CDCl_3$): δ 2.01-2.10 (12H, 4 x OCO$\underline{CH_3}$), 2.50 (3H, Ar-OCO$\underline{CH_3}$), 3.95 (*m*, H-5'), 4.21 (*dd*, *J* = 2.6, 12.4, H-6a'), 4.33 (*dd*, *J* = 5.3, 12.4, H-6b'), 5.2-5.3 (3H, *m*, Glc), 5.32 (*d*, *J* = 7.2, H-1'Glc), 5.39 (2H, lactone CH_2), 7.14 (*d*, *J* = 7.3, H-7), 7.56 (*t*, *J* = 7.69, H-6), 7.64 (*d*, *J* = 7.5, H-5), 7.72 (*br s*, H-4); ^{13}C NMR See Table 1.

Methylation of geshoidin (6). 200 mg of geshoidin in 2 mL DMF was methylated with Ag_2O (2 eq. per OH group, 613.8 mg) and methyl iodide (3 eq. per OH, 0.5 mL) at r.t. for 24 h The reaction mixture was filtered and the filtrate diluted with water and then buffered with solid KCN. This was extracted three times, washed with water and dried. This led to 59.6 mg of the permethylated geshoidin, mp 154-155° yellow brown crystals, light green with vanillin-H_2SO_4, ^1H NMR (300 MHz, $CDCl_3$): δ 7.05 (*dd*, *J* = 2.8, 6.1, H-7), 7.49 - 7.52 (*m*, H-5 and H-6), 7.49 (*s*, H-4), 5.34 (*br s*, lactone CH_2), 5.13 (*d*, *J* = 7.5, H-1' Glc), 4.17 (ArOMe), 3.74, 3.70, 3.58 and 3.35 (4 x OMe); ^{13}C NMR (22.5 MHz, $CDCl_3$): δ 157.97 (C-1 or C-8), 116.8 (C-2), 114.2 (C-3 or C-4a), 118.9 (C-4), 139.9 (C-4a or C-3), 116.3 (C-5), 131.1 (C-6), 111.5 (C-7), 156.6 (C-8 or C-1), 110.4 (C-8a), 167.96 (C=O), 68.7 (lactone CH_2), 90.7 (C-1'), 82.1 (C-2'), 83.2 (C-3'), 79.6 (C-4'), 71.5 (C-5'), 70.0 (C-6'), 58.7, 59.1, 60.2, 60.6 (OCH_3) at 2',3',4' and 6', respectively, 64.9 (1-OMe).

Hydrolysis of geshoidin (6). 50 mg of geshoidin in 10 mL of MeOH-H_2SO_4 (3 N), 7:3, was refluxed for 10 h The methanol was removed on a rotary evaporator and 2 mL of cold water added. The precipitate was extracted with dichloromethane and faint yellow needles of the aglycone (**8**). The washings of the above hydrolysis product were neutralized with potassium carbonate and the liquid filtrate was concentrated to half its volume. Paper chromatography using n-BuOH-HOAc-H_2O 4:1:5), upper layer and spraying with aniline hydrogen phthalate using glucose as reference confirmed the presence of this sugar in the hydrolysate.

Hydrolysis of permethylgeshoidin. 50 mg of permethylgeshoidin was dissolved in 4 mL of methanol and 2 mL of 6% HCl added to the solution. The mixture was refluxed for 8 h and extracted with $CHCl_3$. Removal of solvent led to the isolation of ß-sorigenin-1-methylether, light brown solid, mp 215-217; ^1H NMR (300 MHz, $CDCl_3$): δ 7.53 (*s*, H-4), 7.52 (*t*, *J* = 7.9, H-6), 7.35 (*br d*, *J* = 7.7, H-5), 6.96 (*d*, *J* = 7.7, H-7), 5.39 (*s*, CH_2), 4.45 (*s*, OMe), 9.76 (*s*, OH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.22, 156.00, 156.54, 141.24, 139.89, 131.26, 119.07, 116.83, 116.46, 111.54, 110.49, 68.91, 65.16.

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