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Abstract

Two new dihydroisocoumarin glucosides have been isolated from the stem bark of *Caryocar glabrum* (Aubl.) Pers. Their structures and absolute stereochemistry were established on the basis of extensive 1D and 2D NMR, UV, IR, HRESIMS, and CD studies. These compounds represent the first members of a new biogenetic pathway for the isocoumarins nucleus involving shikimate derived A-ring coupling with a phenyl propanoid derivative.

Keywords: *Caryocar glabrum*; Caryocaraceae; Dihydroisocoumarin.

1. Introduction

Isocoumarins are secondary metabolites structurally related to the coumarins, but with an inverted lactone ring (Hill, 1986; Devienne et al., 2002). They are extremely variable in structure, due to the various types of substitution in their basic skeleton, which can influence their biological activity (Hill, 1986). Isocoumarins have been isolated from a diverse range of natural organisms that includes, *inter alia*, the fungi, plants, and insects (Hill, 1986; Devienne et al., 2002). Isocoumarin itself has not been found to occur naturally, but simple derivatives abound in nature; many of these have substituents at C-3. Most of the natural isocoumarins are derived biosynthetically from the acetate-malonate pathway by condensation of a polyketomethylene chain (Hill, 1986; Kurosaki et al., 1989). Loss of the oxygen function at C-6 of an isocoumarin is quite common, but loss of the hydroxyl group at C-8 never occurs in isocoumarins derived from the acetate pathway, and further oxygenation or alkylation may occur at the remaining positions (Hill, 1986; Goswami et al., 2005). Isocoumarins which lack oxygenation at C-8 are found in plants and are derived biogenetically from the shikimate pathway (Hill, 1986). The 3,4-dihydroisocoumarins are also formed by condensation of the polyketomethylene chain after the reduction of its fifth carbonyl group before the lactone ring closure (Kurosaki, 1996; Kurosaki et al., 1999). The 3,4-dihydroisocoumarins are often found together with their corresponding isocoumarins (Hill, 1986).

The isocoumarins display a very wide range of biological activities including: antifungal, antiviral, antimicrobial, antimalarial, phytotoxic, diuretic, antihypertensive, antitumoral and antileukemic (Hill, 1986; Kurosaki et al., 2005).

Caryocar glabrum (Aubl.) Pers. (Caryocaraceae) is a large tree growing in northern Amazonia, and is used in traditional medicine for the treatment of skin problems (Prance, 1987; Grenand et al., 2004). Previous chemical studies of its fruits demonstrated the presence of triterpenoid saponins (Alabdul Magid et al., 2006). In continuation of studies on this species, an investigation of the stem bark led to the isolation of two new glucosides (**1**-**2**), from the methanol extract of the stem bark of *C. glabrum.* These compounds represent the first members of a new biogenetic pathway for the isocoumarins nucleus involving shikimate derived A-ring (gallic acid) coupling with a phenyl propanoid derivative. The present paper reports the isolation and structure elucidation of these compounds; their stereochemistry was established from analysis of their NMR data and by measurement of CD spectra.

2. Results and discussion

The MeOH extract of the air-dried, stem bark of *C. glabrum* was partitioned successively between H_2O/hexane, and H_2O/EtOAc. The H_2O-soluble fraction was first fractionated by Sephadex LH-20 column chromatography and the resulting fractions were purified by C_{18} column chromatography and semi-preparative HPLC to afford **1** (0.004%) and **2** (0.006%) (Fig.1).

Compound **1** was isolated as a brown powder with a positive optical rotation ($[\alpha]_D^{20}$+ 15). The IR spectrum of **1** showed absorption bands for hydroxyl groups (3429 cm^{-1}), aromatic rings (1618, 1519, and 1459 cm^{-1}), and a lactone carbonyl group (1680 cm^{-1}). The positive HRESIMS of **1** displayed a molecular ion peak $[M + Na]^+$ at *m/z* 533.1273, suggesting the molecular formula $C_{23}H_{26}O_{13}Na$ and 11 degrees of unsaturation in the molecule. Positive ESIMS-MS experiments on its molecular ion $[M + Na]^+$ gave an ion

fragment at *m/z* 371, attributed to the loss of a hexose unit $[M + Na - 162]^+$. Consequently, compound **1** was a monoglycoside, as shown by the presence in its ^{13}C NMR spectrum of a signal at δ_C 104.6 which correlated in the HSQC spectrum with an anomeric proton signal at δ_H 4.32 (*d*, *J* = 7.8 Hz). The COSY and HSQC experiments allowed the complete assignment of all protons and carbons of the sugar which was identified as a β-D-glucopyranose unit on the basis of the large coupling constants (> 7.8 Hz) between H-1", H-2", H-3", H-4", and H-5" (Table 1), and by the measurement of its optical rotation after acid hydrolysis of the methanol extract. Further analysis of the ^1H NMR and COSY spectra revealed the presence of two aromatic rings. The first was 1,3,4-trisubstituted (C-ring) with three protons resonating at δ_H 6.81 (*d*, *J* = 1.9 Hz, H-2'), 6.70 (*d*, *J* = 8.2 Hz, H-5'), and 6.50 (*dd*, *J* = 8.2, 1.9 Hz, H-6'), and the second was penta-substituted (A-ring) with a single proton resonating at δ_H 7.16 (*s*, H-8). A four spin system -CH-CH(O)-CH_2(O)- was observed at δ_H 4.58 (*brs*, H-4), 4.89 (*td*, *J* = 6.6, 1 Hz, H-3), 3.97 (*dd*, *J* = 10.8, 6.6 Hz, H-3a) and 3.83 (*dd*, *J* = 10.8, 6.5 Hz, H-3a), and a methoxyl group was identified at δ_H 3.79 (*s*, 3'-OCH_3) (Table 1). The ^{13}C NMR spectrum of **1** confirmed the presence of the two aromatic rings, the -CH-CH(O)-CH_2(O)- system at δ_C 38.6 (C-4), 84.5 (C-3), 71.1 (C-3a), and the methoxyl group at δ_C 56.3. In addition, the ^{13}C NMR spectrum of **1** revealed that the two aromatic rings contain five oxygenated carbons resonating at δ_C 144.0 (C-5), 141.6 (C-6), 146.3 (C-7), 149.0 (C-3'), and 146.3 (C-4'). The presence of an ester/lactone carbonyl group at δ_C 167.1 (C-1) was also observed (Table 1). The HMBC spectrum of **1** showed $^3J_{H\text{-}C}$ long range correlations between the anomeric proton Glc-H-1"/C-3a, H-3/C-1', H-4/C-2' and C-6', indicating the following structural arrangement Ar-CH-CH(O)-CH_2-O-Glc (Fig. 2). The long range HMBC correlations observed between H-3, H-4 and C-10, and between H-4 and C-5 and C-9, suggested that C-10 was connected to C-4. Together with the correlations of H-8 with C-6 and C-7, these HMBC correlations permitted the assignment of the non-protonated carbons of ring A (Fig. 2). Finally, the last correlations

of H-3 and H-8 with the carbonyl C-1 indicated that **1** contained a 3,4-dihydroisocoumarin core (Fig. 2). The HMBC correlations between H-2', H-6' and C-4', and between H-2', H-5' and C-3', permitted the assignment of the oxygenated carbons of ring C. The $^{3}J_{H\text{-}C}$ correlation between the protons at δ_H 3.79 and C-3' showed that the methoxyl group was attached to C-3'. The above data led to the assignment of **1** as 3-(*β*-D-glucopyranosyloxymethyl)-3,4-dihydro-5,6,7-trihydroxy-4-(4'-hydroxy-3'-methoxyphenyl)-1H-[2]-benzopyran-1-one.

The small value of the vicinal coupling constant $J_{H\text{-}3,H\text{-}4}$ (1 Hz) in **1** suggested a *synclinal* (*sc* or *gauche*) conformation, but did not allow a distinction between the *cis* (axial-equatorial) and *trans* (equatorial-equatorial) configuration of H-3 and H-4 (Hutchings et al., 1991; Bogdanov et al., 2004). The NOESY spectrum of **1** showed NOE effects between H-3/H-4, H-3/H-2', H-3/H-6', and H-4/H-3a, indicating that the *β*-D-glucopyranosyloxymethyl group at C-3 and the 4′-hydroxy-3′-methoxy-phenyl group at C-4 were in a *trans*-diaxial orientation (Fig. 3). Thus, the relative configuration at C-3 and C-4 in **1** was confirmed to be *trans* with an equatorial-equatorial arrangement for H-3 and H-4.

Dihydroisocoumarins possess a benzoic ester chromophore, whose chiroptical properties have been systematically investigated. It was found that the sign of the Cotton effect of this chromophore system could be quite safely used for establishing the absolute conformation of the heteroring B (Krohn et al., 1997). The sign of the Cotton effect of n → π* origin is independent of the substitution pattern of the aromatic ring system (Krohn et al., 1997). The CD spectrum of **1** showed a positive Cotton effect at 234 nm, a negative Cotton effect at 245 nm, and a positive Cotton effect at 288 nm. In accordance with published CD data for 3,4-dihydroisocoumarins, the positive Cotton effect at 288 nm is assignable to the n → π* transition of the carbonyl group of the dihydroisocoumarin chromophore. This indicated that the *β*-D-glucopyranosyloxymethyl was in an *α-axial* orientation, and accordingly the absolute configuration at C-3 of **1** was concluded to be *S* (Hashimoto et al.,

1987; Speranza et al., 1993; Krohn et al., 1997; Ito et al., 2000; Kurosaki et al., 2005; Zidorn et al., 2005). This result indicated that the C-ring was in a *β-axial* orientation and the absolute configuration at C-4 was also concluded to be *S*. On the basis of the foregoing evidence, compound **1** was elucidated as (3*S*,4*S*) 3-(*β*-D-glucopyranosyloxymethyl)-3,4-dihydro-5,6,7-trihydroxy-4-(4'-hydroxy-3'-methoxyphenyl)-1H-[2]-benzopyran-1-one.

Compound **2** was isolated as a brown powder with a positive optical rotation ($[\alpha]_D^{20}$+ 15.6). The IR spectrum showed, as in **1**, the presence of hydroxyl groups (3427 cm^{-1}), aromatic rings (1620, 1518, and 1455 cm^{-1}), and a lactone carbonyl group (1670 cm^{-1}).The positive HRESIMS of **2** displayed a molecular ion peak $[M + Na]^+$ at *m/z* 563.1371 in accordance with the molecular formula $C_{24}H_{28}O_{14}Na$. As in **1**, positive ESIMS-MS experiments on its molecular ion $[M + Na]^+$, gave an ion fragment at *m/z* 401, attributed to the loss of a hexose unit $[M + Na - 162]^+$. The spectral features indicated that **2** was closely related to **1**, in which a 3,4-dihydroisocoumarin was also substituted in position-3 by a *β*-D-glucopyranosyloxymethyl, and in position-4 by an aromatic ring. Comparison of the NMR data of **2** with **1** showed that **2** contained two methoxyl groups at δ_H 3.76 (s, 6H) and δ_C 56.7 (2C), arranged symmetrically (Table 1). The superposition of the chemical shifts of C-2'/C-6' and of C-3'/C-5', as well as the HMBC correlations of the two proton singlet at δ_H 6.44 (H-2' and H-6') with C-1' and C-4, indicated that the aromatic C-ring was 3,4,5-trioxy substituted. The HMBC correlations of the methoxyl groups with C-3'/C-5' at δ_C 149.2 showed that they were linked to C-3' and C-5' of C-ring. Comparison of the NOESY and CD data of **2** and **1** showed that the relative configuration of **2** is 3,4-*trans* and its absolute configuration is 3*S*, 4*S*, as in **1**. Thus, compound **2** was concluded to be (3*S*,4*S*) 3-(*β*-D-glucopyranosyloxymethyl)-3,4-dihydro-5,6,7-trihydroxy-4-(4'-hydroxy-3'-5′-dimethoxyphenyl)-1H-[2]-benzopyran-1-one.

In order to verify that **1** and **2** were natural and not artefactual products, the stem bark was extracted with cold methanol and then, the methanol extract was partitioned as previously described. The H_2O-soluble fraction was analysed by analytical HPLC and the chromatogram showed the presence of the two compounds **1** and **2** confirming their natural origin.

From a biogenetic viewpoint, isocoumarins are usually polyketide in origin and result from the acetate-malonate pathway through cyclization reactions at the end(s) of a polyketide chain, and they are normally substituted on position 3 and hydroxylated at position 8 (Hill, 1986). For compounds **1** and **2**, there seem to be no doubt that they arise from the addition of gallic acid to coniferyl alcohol (or coniferyl alcohol glucoside) for **1** and of gallic acid to sinapyl alcohol (or sinapyl alcohol glucoside) for **2**. More likely, the first step in the formation is the linkage between the gallic acid moiety at the 2-position, followed by cyclization to form the lactone nucleus. Compounds **1** and **2** represent the first members of a new biogenetic pathway for the isocoumarins nucleus involving shikimate derived A-ring coupling with a phenyl propanoid derivative. Therefore, these compounds could be important from the viewpoint of isocoumarin metabolism in the plant kingdom.

3. Experimental

3. 1. General experimental procedures

Optical rotations were measured in MeOH or H_2O with a Perkin-Elmer 241 polarimeter. UV spectra were obtained using a Philips PU 8720 spectrophotometer. CD spectra were recorded with a Jasco J-810 spectropolarimeter. IR spectra were recorded on a Nicolet Avatar 320 FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded in CD_3OD on a Bruker Avance DRX-500 spectrometer (^{1}H at 500 MHz and ^{13}C at 125 MHz), and 2D-

NMR experiments were performed using Bruker's standard microprograms (XWIN-NMR version 2.6 software). ESIMS and HRESIMS experiments were performed using a Micromass Q-TOF micro instrument (Manchester, UK). TLC was carried out on precoated silica gel 60 F_{254} (Merck), with $CHCl_3$-MeOH-H_2O (13:7:1), and spots were visualized by heating after spraying with 50% H_2SO_4. Column chromatography was carried out on Sephadex LH-20 (Pharmacia) and LiChroprep RP-C_{18} (40 - 63 µm, Merck). HPLC was performed on a Dionex apparatus equipped with an ASI-100 autosampler, a STH 585 column oven, a P580 pump, a UVD 340S diode array detector, and the Chromeleon software. A prepacked RP-C_{18} column (Thermo Electron corporation hyperprep HS, 21.2 x 250 mm, 10µ) was used for semi-preparative HPLC with a flow rate of 5 $ml.min^{-1}$ and the chromatogram was monitored at 270 nm. A RP-C_{18} column (201 SPTM, 4.6x250 mm, 5µ, 90 Å Dionex, vydac, France) was used for analytical HPLC with an isocratic elution (MeCN 10% in H_2O/ pH 2.4 with 0.0025 % TFA), a flow rate of 1 $ml.min^{-1}$ and the chromatogram was monitored at 270 nm.

3.2. Plant material

The stem bark of *C. glabrum* was collected in the Amirande forest in French Guyana, at Saint Georges de l'Oyapock station in October 2001 and March 2007. The species was identified by M.F. Prevost of the Botany Laboratory of the IRD Cayena (French Guyana), and a herbarium specimen (EC-168) was deposited in the Herbier of Guyana.

3.3. Extraction and isolation

The powdered, air-dried stem bark (396 g) was boiled under reflux in methanol (1 l) for 3 h to provide a brown pasty residue (29 g; yield 7.3%). This residue was suspended in

H_2O (200 ml) and partitioned between H_2O and hexane (3 x 200 ml), affording a hexane soluble fraction (190 mg). The resulting H_2O-soluble fraction was partitioned between H_2O and EtOAc (3 x 200 ml) affording an EtOAc-soluble fraction (13 g) and a H_2O-soluble fraction (15 g). A part of the H_2O-soluble fraction (14 g) was dissolved in H_2O and applied to a Sephadex LH-20 column (2.7 x 70 cm), eluting successively with H_2O, 50% and 80% MeOH-H_2O, and finally with MeOH. Fractions eluted with 80% MeOH-H_2O were purified by column chromatography over C_{18} silica gel using a gradient of MeOH-H_2O (3:7 to 8:2). Fractions eluted with MeOH-H_2O (3:7) were further purified by semi-preparative HPLC, with an isocratic elution [CH_3CN-H_2O-TFA (10:90:0.0025)] to give **1** (17 mg, 0.004 %) and **2** (25 mg, 0.006 %).

3.4. Acid hydrolysis

A sample methanol extract (100 mg) of the stem bark was refluxed with 2N HCl (15 ml) for 4h 30 min. The mixture was extracted with EtOAc (3 x 15 ml), and the acidic aqueous layer was neutralised with 0.5 N KOH and freeze-dried. Glucose was identified by TLC with authentic sugar samples in MeCOEt - *iso*-PrOH - Me_2CO - H_2O (20:10:7:6). After preparative TLC of the sugar mixture (100 mg) in this solvent, measurement of the optical rotation indicated the presence of a D-glucose [$[\alpha]_D^{20}$ + 11° (H_2O; *c* 0.12)].

*3. 5. Compound **1***

Amorphous brown powder; $[\alpha]_D^{20}$: + 15 (MeOH; *c* 0.57); UV λ_{max} (MeOH): 221 (1.4), 281 (0.5) nm (log ε); IR (KBr) υ_{max}: 3429, 1680, 1618, 1519, 1459, 1384, 1330, 1203, 1111, 1073 CM^{-1}; CD (MeOH): $\Delta\varepsilon_{234}$ +1.89, $\Delta\varepsilon_{245}$ - 1.02, $\Delta\varepsilon_{288}$ + 0.94 (*c* 1.96 x 10^{-7} M); ^1H and ^{13}C

NMR (CD_3OD) see Table 1; ESIMS (positive ion mode): 533 $[M + Na]^+$, ESIMS-MS (533) *m/z*: 371 $[M + Na - Glc]^+$; HRESIMS (positive ion mode) *m/z*: 533.1273 $[M+Na]^+$ (calcd. for $C_{23}H_{26}O_{13}Na$, 533.1271).

*3. 6. Compound **2***

Amorphous brown powder; $[\alpha]_D^{20}$: + 15.6 (MeOH; *c* 0.57); UV λ_{max} (MeOH): 233 (0.8), 283 (0.4) nm (log ε); IR (KBr) υ_{max} 3427, 1703, 1670, 1620, 1518, 1455, 1379, 1333, 1241, 1200, 1115, 1075, 1036 CM^{-1}; CD (MeOH): $\Delta\varepsilon_{235}$ + 0.60, $\Delta\varepsilon_{245}$ - 1.80, $\Delta\varepsilon_{295}$ + 0.32 (*c* 1.85 x 10^{-7} M); ^1H and ^{13}C NMR (CD_3OD) see Table 1; ESIMS (positive ion mode): 563 $[M + Na]^+$, ESIMS-MS (563) *m/z*: 401 $[M + Na - Glc]^+$; HRESIMS (positive ion mode) *m/z*: 563.1371 $[M+Na]^+$ (calcd. for $C_{24}H_{28}O_{14}Na$, 563.1377).

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References

Alabdul Magid, A., Voutquenne, L., Moretti, C., Long, C., Lavaud, C., 2006. Triterpenoid saponins from the fruit of *Caryocar glabrum.* J. Nat. Prod. 69, 196 - 205.

Bogdanov, M.G., Todorov, I.S., Manolova, P.G., Cheshmedzhieva, D.V., Palamareva, M.D., 2004. Configuration and conformational equilibrium of (±)-*trans*-1-oxo-3-thiophen-2-yl-isochroman-4-carboxylic acid methyl ester. Tetrahedron Lett. 45, 8383–8386.

Devienne, K.F., Raddi, M. S.G., Varanda E.A., Vilegas, W., 2002. *In vitro* cytotoxicity of some natural and semi-synthetic isocoumarins from *Paepalanthus bromelioides*. Z. Naturforsch. 57c**,** 85-88.

Goswami, S., Gupta, V.K., Brahmbhatt, D.I., Pandya, U.R., 2005. Crystal structure and synthesis of 3-benzyl-3-phenyl-3,4-dihydroisocoumarin. Anal. Sci. 21, 213-214.

Grenand, P., Moretti, C., Jacquemin, H., Prévost, M.F., 2004. Pharmacopées Traditionnelles en Guyane, Créoles, Wayasi, Palikur. IRD, Paris.

Hashimoto, T., Tori, M., Asakawa, Y., 1987. Three dihydroisocoumarin glucosides from *Hydrangea macrophylla* subsp. *serrata*. Phytochemistry 26, 3323-3330.

Hill, R.A., 1986. Naturally occuring isocoumarins. Herz, W., Grisebach, H., Kirby, G.W., Tamm, C. (Eds.), Progress in the Chemistry of Natural Products, Vol. 49. Springer Verlag, New York, pp 1-78.

Hutchings, M.G., Chippendale, A.M., Shukla, R., McPartlin, M., 1991. Alkoxide-induced ring-opening of methoxymethylidene-substituted homophthalic anhydride Part 2. Structure and conformational analysis of the dihydroisocoumarin products. Tetrahedron 47, 7869-7874.

Ito, C., Mishina, Y., Litaudon, M., Cosson, J.P., Furkawa, H., 2000. Xanthone and dihydroisocoumarin from *Montrouziera sphaeroidea*. Phytochemistry 53, 1043-1046.

Krohn, K., Bahramsari, R., Florke, U., Ludewig, K., Kliche-Spory, C., Michel, A., Aust, H.-J., Draeger, S., Schulz, B., Antus, S., 1997. Dihydroisocoumarins from fungi: isolation, structure elucidation, circular dichroism and biological activity. Phytochemistry 45, 313-320.

Kurosaki, F., 1995. Dissociation of dimeric 6-hydroxymellein synthase, a polyketide biosynthetic enzyme in carrot cell extracts, with loss of ketoreducing activity. Arch. Biochem. Biophys. 321, 239–244.

Kurosaki, F., Kizawa, Y., Nishi, A., 1989. Biosynthesis of dihydroisocoumarin by extracts of elicitor-treated carrot root. Phytochemistry 28, 1843-1845.

Kurosaki, F., Togashi, K., Arisawa, M., 2000. Role of reducing cofactors in catalytic reactions of 6-hydroxymellein synthase, a multifunctional polyketide biosynthetic enzyme in carrot cells. Plant Sci. 160, 113–120.

Kurosaki,Y., Fukuda, T., Iwao, M., 2005. Asymmetric synthesis of 3-substituted 3,4-dihydroisocoumarins via stereoselective addition of laterally lithiated chiral 2-(*O*-tolyl) oxazolines to aldehydes followed by diastereomer-selective lactonization. Tetrahedron 61, 3289-3303.

Prance, G.T., 1987. An updata on the taxonomy and distribution of the Caryocaraceae. Opera Bota. 92, 179-184.

Speranza, G., Manitto, P., Cassara, P., Monti, D., 1993. Feralolide, a dihydroisocoumarin from *Cape aloe*. Phytochemistry 33, 175-178.

Zidorn, C., Lohwasser, U., Pschorr, S., Salvenmoser, D., Organia, K.-H., Ellmerer, E.P., Börner, A., Stuppner, H. 2005. Bibenzyls and dihydroisocoumarins from white salsify (*Tragopogon porrifolius* subsp. *porrifolius*). Phytochemistry 66, 1691-1697.

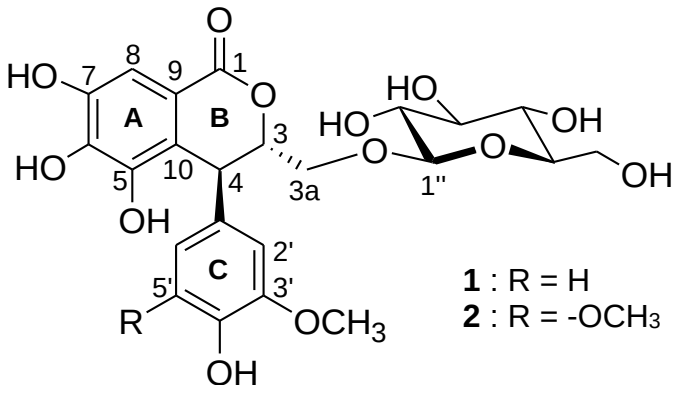

Figure 1. Structures of compounds **1** and **2**.

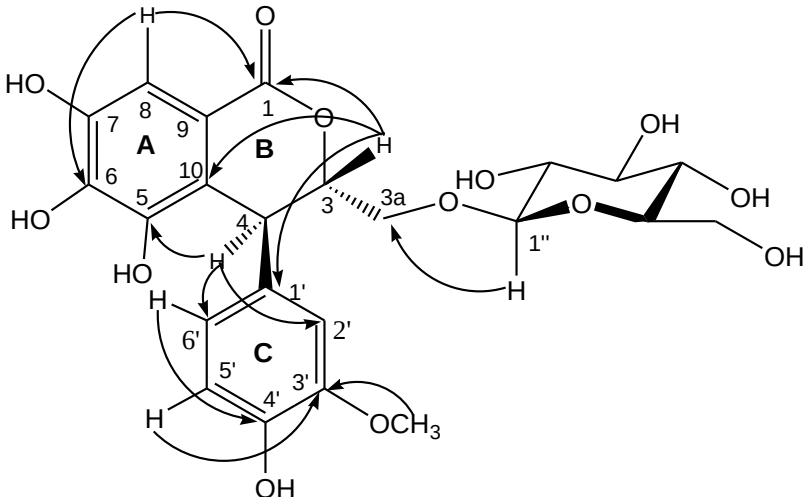

Figure 2. Key $^{3}J_{H\text{-}C}$ HMBC correlations of compound **1**.

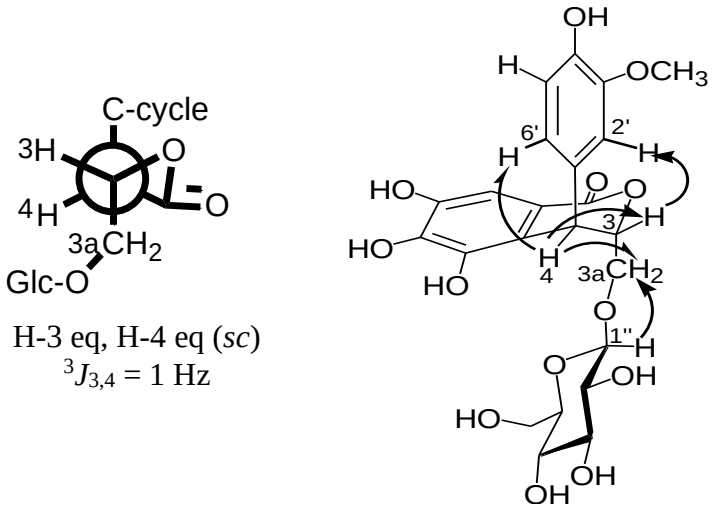

Figure 3. Conformation and selected NOEs of compound **1**.

Table 1. ^{1}H and ^{13}C NMR spectral data of **1** and **2** (in CD_3OD).

	1		**2**	
	δ_H (*m*, *J* Hz)	δ_C	δ_H (*m*, *J* Hz)	δ_C
Aglycone				
1	-	167.1	-	167.1
3	4.89 (*td*, 6.6, 1)	84.5	4.90 (*td*, 6.6, 1.1)	84.5
3a	3.83 (*dd*, 10.8, 6.5)	71.1	3.82 (*dd*, 10.9, 6.3)	71.0
	3.97 (*dd*, 10.8, 6.6)		3.95 (*dd*, 10.9, 6.8)	
4	4.58 (*brs*)	38.6	4.59 (*brs*)	38.9
5	-	144.0	-	144.1
6	-	141.6	-	141.6
7	-	146.3	-	146.4
8	7.16 (*s*)	108.8	7.17 (*s*)	108.8
9	-	115.9	-	115.9
10	-	121.2	-	120.9
1′	-	134.5	-	133.8
2′	6.81 (*d*, 1.9)	112.6	6.44 (*s*)	106.2
3′	-	149.0	-	149.2
4′	-	146.3	-	135.4
5′	6.70 (*d*, 8.2)	116.0	-	149.2
6′	6.50 (*dd*, 8.2, 1.9)	121.2	6.44 (*s*)	106.2
3′-OCH$_3$	3.79 (*s*)	56.3	3.76 (*s*)	56.7
5′-OCH$_3$	-	-	3.76 (*s*)	56.7
β-D-glucopyranose				
1″	4.32 (*d*, 7.8)	104.6	4.32 (*d*, 7.8)	104.6
2″	3.22 (*dd*, 8.8, 7.8)	75.0	3.22 (*dd*, 8.9, 7.8)	75.0
3″	3.37 (*t*, 8.8)	77.9	3.37 (*t*, 8.9)	77.9
4″	3.32 (*t*, 8.7)	71.4	3.31 (*t*, 8.6)	71.4
5″	3.26 (*ddd*, 8.7, 5.4, 2.2)	77.9	3.27 (*ddd*, 8.6, 5.4, 2.2)	77.9
6″a	3.67 (*dd*, 12.5, 5.4)	62.6	3.67 (*dd*, 11.9, 5.4)	62.6
6″b	3.85 (*dd*, 12, 2.2)		3.85 (*dd*, 12, 2.2)	

Dihydroisocoumarin glucosides from stem bark of *Caryocar glabrum*

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Two dihydroisocoumarin glucosides, **(1**-**2**), were isolated from the stem bark of *Caryocar glabrum*. The aglycone part is a new dihydroisocoumarin skeleton arising from the condensation of gallic acid with a phenylpropanoid derivative.