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Fluorescent Pigment and Phenol Glucosides from the Heartwood of *Pterocarpus marsupium*

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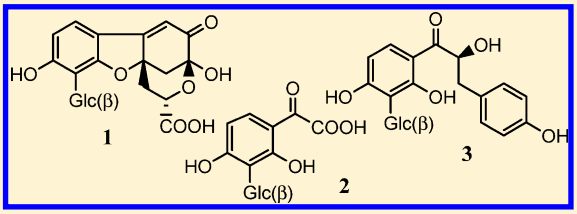
Supporting Information

ABSTRACT: The fluorescence shown by extracts of the heartwood of *Pterocarpus marsupium* is attributed to salts of the new compound **1**, whose structure was elaborated using detailed spectroscopic/spectrometric studies. The plant material also contains the nonfluorescent compounds **2** and **3**. The absolute configuration of **1** was determined by experimental and theoretically calculated electronic CD spectra, while that of **3** was deduced from ECD comparison with reported results in the α-hydroxydihydrochalcone series.



The heartwood of *Pterocarpus marsupium* Roxb. (Fabaceae) is endowed with various medicinal properties,[1] and these features are revealed in recent patents[2] for herbal preparations useful in diabetes. The known constituents represent various flavonoids, diphenylpropane derivatives, and sesquiterpenes. Since flavone glycosides and plant extracts rich in this class of compounds are suggested to be useful for therapy and prophylaxis of diabetes,[3] similar constituents of this plant have been under intense investigation.[4−6]

Our primary interest was, however, in the polar constituents of the plant, as the heartwood has been reported[7] to contain an uncharacterized water-soluble fluorescent material. Fluorescence spectroscopy is regarded as an important tool for the identification of timbers. Indeed a study on the fluorescence characteristics of the dry wood of this plant and its methanolic extract showed distinctive differences, suggesting[7] a detailed examination of the pure constituent responsible for the fluorescence phenomenon. However, isolation of the fluorescent constituent has not been reported by earlier investigators, except for a preliminary finding.[8] Purification through a combination of Biogel P-2 chromatography and preparative paper chromatography of the crude extract yielded a sample exhibiting an emission maximum at 467 nm with an excitation maximum at 428 nm. Its mass and NMR spectra suggested it to be a phenolic glycoside of molecular weight 466 having an aliphatic appendage. It was, therefore, necessary to develop a more convenient method of purification to obtain the material in sufficient quantities for proper structural investigation. The details of this study are described herein.

RESULTS AND DISCUSSION

The heartwood of *P. marsupium* was extracted with MeOH. The dried MeOH extract was washed with EtOH and then re-extracted with MeOH to concentrate the fluorescent compound(s). Repeated chromatography of this over Diaion HP-20 yielded nonfluorescent material in the initial fractions eluted with H_2O and the fluorescent material in subsequent fractions. Further elution with 20−30% MeOH in H_2O produced a brown solution showing the same fluorescent spot on TLC and containing **1**. Rechromatography of the initial fractions over silica gel afforded compound **2**. Fractions eluted with 50% aqueous MeOH from the Diaion column, after further chromatography on silica gel, yielded compound **3**.

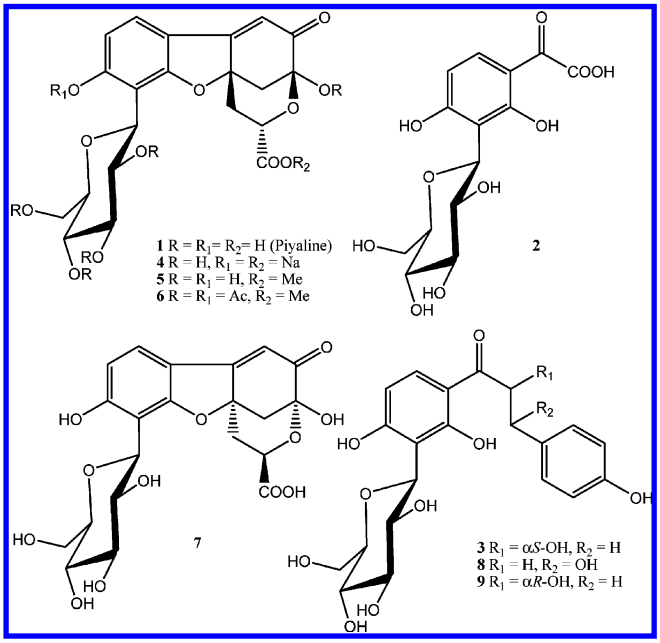


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Table 1. ^{13}C and ^{1}H NMR Data (in D_2O; 5 in Methanol-d_4)

	δ_C				δ_H mult (J in Hz)		
C no. (type)	7[a]	1	4	5	1	4	5
1 (C)	89.0	88.2	88.8	89.2			
2 (CH_2)	44.5	44.1	44.2	45.9	2.60, d (11.4); 2.54, d (11.4)	2.55, d (11); 2.44, d (10)	2.54, d (10.8); 2.41, d (10.8)
3 (C)	94.9	94.6	94.7	96.0			
4 (C)	191.5	192.8	190.6	192.9			
5 (CH)	106.7	111.2	105.2	112.1	6.46, d (1.2)	6.22, s	6.36, s
6 (C)	168.9	167.1	169.0	167.4			
1′ (C)	109.8	113.8	108.4	114.6			
2′ (C)	167.8	165.2	168.1	166.9			
3′ (C)	107.5	107.2	107.2	110.4			
4′ (C)	167.8	163.0	175.0[b]	165.6			
5′ (CH)	117.7	112.6	118.7	113.8	6.74, d (8.4)	6.45, d (8.5)	6.64, d (7.2)
6′ (CH)	126.8	126.8	126.5	126.8	7.63, dd (1.5, 8.7)	7.48, d (8.5)	7.53, d (7.8)
1″ (CH)	74.4	74.0	74.2	75.9	4.75, d (10.2)	4.68, d (10)	4.71, d (10.2)
2″ (CH)	70.7	70.7	70.3	72.9	4.16, t (9)	4.31, m	4.04, t (9)
3″ (CH)	78.2	77.5	78.1	80.0	3.5−3.6, m	3.53−3.60, m	3.40−3.49, m
4″ (CH)	69.8	69.6	69.5	71.9	3.5−3.6, m	3.53−3.60, m	3.40−3.49, m
5″ (CH)	80.6	80.6	80.3	82.6	3.5−3.6, m	3.53−3.60, m	3.40−3.49, m
6″ (CH_2)	61.2	60.8	60.9	63.0	3.89, d (12); 3.78, dd (4.5, 12.3)	3.87, d (12); 3.77, br d	3.87, br d (12); ∼3.70
α (CH)	70.0	68.4	69.7	69.2	4.41, dd (3.3, 12.3)	4.31, m	4.48, dd (4,13)
β (CH_2)	35.9	34.5	35.8	36.1	2.21, t (13); 2.05, br d (∼12.6)	2.13, t (12.2); 1.97, br d (12)	2.19, t (13.2); 1.99, br d (∼12.0)
COOR	177.4	175.2	177.3	172.0, 53.1			3.70, s

[a]ref 10. [b]Broad signal, identified from HMBC.

Although TLC showed no difference, arbitrary pooling of the fluorescent fractions from the initial H_2O eluates (orange colored) and the subsequent collections (light-orange colored) indicated that the fractions comprised salts (di, mono; Na^+/K^+) of **1**, as was obvious from the similarity in NMR spectroscopic patterns coupled with some diagnostic changes (*vide infra*). Addition of 0.1 N NaOH to a solution of **1** afforded a pure sample of the di-Na salt **4**. The FAB mass spectrum of **1** showed prominent peaks at m/z 467 ($[M + H]^+$) and m/z 489 ($[M + Na]^+$), which shifted to m/z 511 and 533 in the spectrum of **4**. The molecular formula of **1** was deduced as $C_{21}H_{22}O_{12}$ from HR-ESIMS analysis and was consistent with ^{13}C and ^{1}H NMR (Table 1) evidence.

Treatment of compound **1** with TFA in MeOH furnished the methyl ester **5** based on MS and NMR data (Table 1). Subsequent spectroscopic and spectrometric analyses were performed mainly with compound **5** to avoid ambiguity. The H2BC spectrum facilitated identification (Table S1, Supporting Information) of a β-glucopyranosyl unit. The *C*- rather than an *O*-glucoside was shown by the ^{13}C NMR spectrum, indicating a distinctly upfield shift for the anomeric carbon (δ_C 75.9). The aglycone part contained a pair of *ortho* aromatic methines (δ_C 113.8, 126.8), an isolated aromatic or conjugated olefinic methine (δ_C 112.1), a $-CH_2-CHO(H/R)-$ moiety (δ_C 36.1, 69.2), and an isolated methylene group (δ_C 45.9) whose protons underwent slow exchange in D_2O, along with broadening of the corresponding ^{13}C NMR resonance.

Examination of the HMBC data of **5** along with the 1,1-ADEQUATE information (Table S1, Supporting Information) permitted the identification of a 1,2,3,4-tetrasubstituted aromatic ring carrying a free OH and a glucopyranosyl moiety, common to many of the congeners, as well as the structural unit **A** (Figure 1). The presence of a trisubstituted double bond was inferred from the occurrence of two downfield carbon resonances (δ_C 112.1 and 167.4). The ADEQUATE experiment also indicated that the other carbon linked to the olefinic

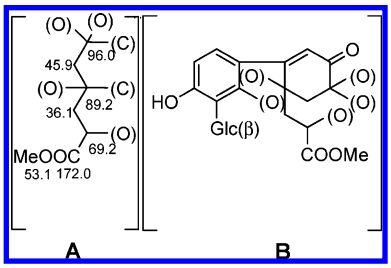

Figure 1. Structural fragments **A** and **B** for **1**.

CH must be either the quaternary carbon, δ_C 96.0, or the α,β-unsaturated carbonyl carbon, δ_C 192.9. That the linkage is to the carbonyl carbon and then to the nonprotonated carbon became obvious from the INADEQUATE spectrum (Table S1), which further revealed a correlation involving the δ_C 89.2 and 167.4 resonances.

Initially it appeared that the olefinic carbon signal at δ_C 167.4 signified an oxygenated carbon to explain the downfield chemical shift, but this led to structural propositions that could not explain the observed fluorescence. However, relying on the weak NOE relationship between the aromatic proton (H-6′) and the conjugated olefinic proton (H-5) in the ^{1}H NMR spectrum facilitated the formulation of an alternative partial structure **B** (Figure 1) and hence of structure **1** for this unique naturally occurring phenolic compound. The equatorial orientation of the α-carboxy group was indicated by the $^{3}J_{H,H}$ values of 12.3 and 3.3 Hz and confirmed by the theoretically calculated preferred conformations (*vide infra*).

Attempted preparation of derivatives either yielded amorphous products difficult to purify or led to the formation of complex mixtures. However, compound **5** yielded the hexa-*O*-acetyl derivative **6**, which could be chromatographically purified. An interesting feature of its NMR spectrum was the considerable broadening of some signals particularly for nuclei around the glycosidic bond. Literature reports[9] on related

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peracetates suggest that this is due to restricted rotation about the C-linked anomeric bond.

The recent publication of the structure of matlaline (**7**), the fluorescent compound of the Mexican medicinal wood *Lignum nephreticum*, by Acuña et al.[10] based, *inter alia*, on the observed oxidative conversion of the co-occurring known compound coatline B suggested that our fluorescent compound must be identical to or stereoisomeric with it. The NMR data of **7** closely match those of the disodio salt **4**, and the carbon chemical shifts of carbons *ortho*, *para*, and vinylogous to *para* (with respect to the phenolic group) compared[11] to those of **1** follow the trend reported for phenols and their salts. Although the signal of the *ipso* carbon relative to the OH group appeared to overlap with that of the other oxygenated carbon, the chemical shift value did not agree with the expected downfield shift. A search in the anticipated region using correlation peaks in the HMBC spectrum suggested that a broad resonance at δ_C 175.0 should preferably be assigned to this carbon. The stereoisomeric nature of **4** and **7** was suggested by the distinct difference in their specific rotations, +32.1 for **7** and +46.0 for **4**. The configuration of matlaline (**7**) was assigned on the basis of its formation from the co-occurring coatline B of known absolute configuration. Compound **1**, therefore, possibly originated from an epimer of coatline B. It is thus a new diastereoisomer[12] of matlaline, named piyaline from the local name of *P. marsupium*.

In order to assist the assignment of the absolute configuration of compound **1**, the electronic circular dichroism (ECD) spectrum was recorded and calculated spectra were generated via time-dependent density functional theory (TDDFT) calculations. The 1*S*,3*S* configuration has been mandatorily designated for the conformational search, affording 60 conformers with an energy cutoff of 20 kcal/mol. Fourteen conformers within 5 kcal/mol were employed for full geometric optimization at the B3LYP/6-31G** level in the gas phase. Eleven conformers were relocated, resulting in three predominant conformers with a Boltzman distribution of 50.8:16.6:32.5%, denoted as **1a**, **1b**, and **1c** in Figure 2 and Table S2,3 and accounting for 99.9% of the

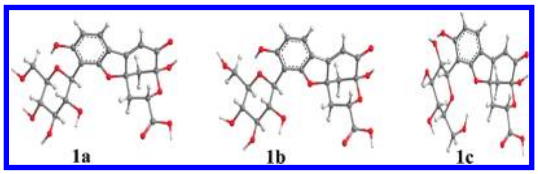

Figure 2. Optimized geometries of predominant conformers of compound **1** at the B3LYP/6-31G** level in the gas phase.

conformational itinerary. The differences between the predominant conformers are the rotations of the glucosyl and hydroxymethylene groups. ECD spectra of predominant conformers have been calculated at the same level in the gas phase.[13]

The experimentally observed and theoretically simulated ECD curves (Figure 3) matched very well. The positive Cotton effect (CE) at 391 nm was reproduced at 373, 371, and 375 nm in the conformers **1a**, **1b**, and **1c**, respectively. The negative CEs at 308 and 345 nm were contributed by the transitions at 304 and 347 nm in **1a**, 306 and 347 nm in **1b**, and 304 and 344 nm in **1c**, respectively. Another high-amplitude negative CE at 230 nm may be attributed to the electronic transitions in the 200−268, 200−267, and 200−269 nm regions in **1a**, **1b**, and **1c**, respectively.

Molecular orbital analysis of conformer **1a** (Table S4 and Figure S1) indicated that the positive rotatory strength at 373 nm could be attributed to the electronic transition from HOMO to LUMO, i.e., the delocalized π orbital involving the coplanar atoms in the benzene, dihydrofuran, and the cyclohexenone rings excluding C-1, C-2, and C-3. The negative CEs at 347 and 304 nm resulted from the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions. The glucosyl-involved transition occurred in the high-energy region at 233 nm. This was confirmed by the ECD calculation of compound **1Me**, in which a methyl group replaced the glucosyl group of **1** (Figure 3); the electronic transitions in the lower energy region remained but shifted to 361, 346, and 307 nm, respectively.

Biogenetically, the new compound may be derived from the α-hydroxydihydrochalcone isomeric with coatline B (*vide supra*) at C-α, via oxidation of the catechol ring followed by Michael addition of the phenolic ring to the *o*-quinone generating a spiro-ring and subsequent steps proposed for matlaline.[10] Acuna et al., using the results of oxidation of the racemic aglycone (synthetic) of matlaline, explained how the configuration at C-α dictates the orientation of the bridged ring.[10] Although coatline B (*vide supra*) or a stereoisomer has not been reported from *P. marsupium* or encountered by us, it is known to contain a deoxy analogue, pterosupin, reported by Adinarayana et al.[14] to be a β-hydroxydihydrochalcone (**8**). Bezuidenhoudt et al.[9] disclosed the presence of the α-hydroxydihydrochalcone coatline A (**9**) in the same source and questioned the β-hydroxydihydrochalcone structure of pterosupin. In order to clinch supporting evidence for the absolute configuration proposed for **1**, we isolated the hydroxydihydrochalcone from *P. marsupium*. Its ^{1}H NMR spectrum in methanol-d_4 was identical with that reported[15] for coatline A. The spectrum recorded in DMSO-d_6 (used in ref 14) also largely agreed with that reported for pterosupin, but two carbon signals in the ^{13}C NMR spectrum differed by >1.5 ppm and the identity could not be confirmed. Interestingly, the specific rotation of our sample (+89.3) was distinctly different from that of coatline A[15] (−45.17). This led us to conclude that our sample may be epimeric to coatline A at the C-α stereogenic center.

The stereochemistry of the α-hydroxydihydrochalcones has been intensively studied by Ferreira and his group.[16] On the basis of ECD studies, they found that for α,2′-dihydroxy-substituted compounds, the (αR)-isomers show a negative Cotton effect near 325 nm and a positive Cotton effect near 245 nm. However, the CD spectra of the glycosylated products proved to be more complex.[9] Subsequently, Alvarez and Delgado isolated[17] a new α-hydroxydihydrochalcone and its xylopyranosides from *Eysenhardtia polystachya* and deduced the absolute configuration of these products and that of the known congener coatline B as αR on the basis of CD data. The C-glycoside showed sequential positive and negative Cotton effects at 290 and 327 nm. The sample isolated by us from *P. marsupium* showed strong positive CEs at 327 and 321 nm, a weaker CE at 291 nm, and a negative CE at 257 nm. Comparing the results with the CD curve of α,2′-dihydroxy-substituted compounds,[16] it may be concluded that the CE near 320 and 250 (rather than 290) nm should be relied upon for deducing the C-α configuration. Thus, our compound must be the (αS)-isomer (**3**), in contrast to the (αR)-isomer, coatline A, isolated from other sources.

Compound **2** had NMR signals reminiscent of those ascribed to the C-linked phenylglucoside part of **1**. The negative ion HR-ESIMS showed a strong $[M - H]^-$ ion peak at *m*/*z*

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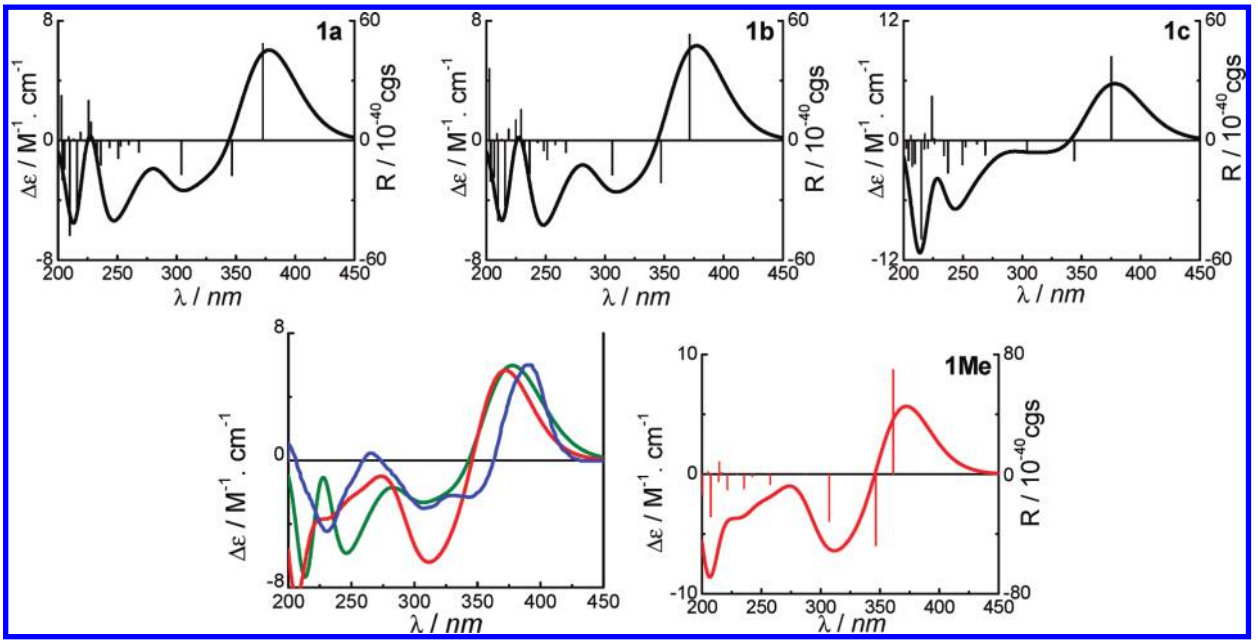

Figure 3. Experimentally observed (blue) and theoretically weighted (olive) ECD of compound **1** and simulated ECD of individual conformers (**1a**, **1b**, **1c**) of compound **1** (black) and of sugar replaced by methyl compound **1Me** (red) at the B3LYP/6-31G** level in the gas phase.

343.0676, in agreement with the molecular formula $C_{14}H_{16}O_{10}$. Although the chemical shifts for the carbonyl groups appear upfield (δ_C 171.5 and 198.1), lower δ values have been reported[17] when such groups are present α to each other, viz., δ_C 207.1, 173.0 for ethyl levulinate, but δ_C 192.0, 160.7 for ethyl pyruvate.

The phenolic glucoside **2** showed a small positive specific rotation (+14.7). The related compounds containing a phenolic β-D-glucoside moiety, e.g., pterocarposide[19] reported from *P. marsupium*, isovitexin (6-glucopyranosyl-4′,5,7-trihydroxyflavone[20]), and genistein-8-*C*-glucoside,[21] show specific rotation values in the range +10 to +25. It therefore appears to be a D-glucoside. Biogenetically, it may be considered as an oxidative degradation product of the congener **3**.

EXPERIMENTAL SECTION

General Experimental Procedures. The following instruments were used for physical data measurements: Jasco P-1020 polarimeter (optical rotations), Shimadzu Pharmaspec 1700 (UV–vis spectra), Jasco J-815 (CD spectra), Bruker DPX 300 and Avance Microbay 600 (NMR spectra), Micromass Q-Tof Micro (ESI-MS), and Jeol JMS700 (FAB-MS). A Waters HPLC instrument consisting of a 515 pump and a 2487 dual absorbance detector was employed for HPLC (both analytical and semipreparative) on reversed-phase C_{18} columns. TLC was performed with Kieselgel 60 F_{254} (Merck). Column chromatography was done using either Diaion HP20 (Supelco) or silica gel 60 (Merck).

Plant Material. The heartwood of *P. marsupium* was collected near Jhargram, West Bengal (India). A herbarium specimen (No. BA/345), deposited at the Central National Herbarium, Botanic Garden, Howrah, West Bengal, was identified by Dr. P. Venu.

Extraction and Isolation. In a typical experiment, the dried and chopped heartwood (1.5 kg) of *P. marsupium* was extracted by soaking in MeOH (3 × 4 L) overnight at room temperature. The combined extract was evaporated to dryness, and the crude extract (50 g) was suspended in EtOH (500 mL) and sonicated. The mother liquor was decanted, the residue was re-extracted twice with EtOH, and the combined mother liquor was evaporated to afford an extract (14 g). The final residue (after EtOH extraction) was extracted by sonication in MeOH (3 × 500 mL). The combined MeOH extract was evaporated to obtain the MeOH extract (17.5 g). A portion (10 g) of the MeOH extract was dissolved in H_2O (with addition of a few drops of MeOH as required) and poured over a column of Diaion HP-20 (300 g, 40 × 3.5 cm). Elution was made successively with H_2O and increasing percentages (10–100) of MeOH in H_2O, and fractions were combined on the basis of TLC (silica gel 60 F_{254}; solvent EtOAc–ethyl methyl ketone–HCO_2H–H_2O, 5:3:1:1, or EtOH–H_2O, 9:1). Initial fractions eluted with H_2O contained **6** (1.7 g), while the later fractions yielded salts, bis (0.5 g) followed by mono (2.5 g), of **1**. Elution with 20–30% MeOH in H_2O gave 1.0 g of **1**. Finally, elution with 1:1 MeOH–H_2O furnished **3** (2.03 g). Fractions were separately rechromatographed on Diaion columns following essentially the same procedure to obtain purer fractions of **2** (1.4 g), di (0.25 g)- and mono (1.9 g)-salts of **1**, **1** itself (0.5 g), and **3** (0.26 g).

The fraction containing compound **1** (0.15 g) was further purified by chromatography on silica gel (9 g) after preadsorption from an aqueous solution. Elution was with EtOAc followed by increasing percentages (10–100%) of MeOH in EtOAc. The purified material (0.1 g) was eluted in 20–40% MeOH in EtOAc fractions.

The fraction containing compound **2** (0.5 g) was similarly chromatographed over silica gel (15 g) to obtain a pure sample (0.32 g) in 30–50% MeOH in EtOAc fractions.

The fraction containing compound **3** (0.26 g) was further chromatographed over silica gel (10 g) to get 0.116 g of a sample, which was subjected to preparative HPLC on an ODS column eluting with MeOH–H_2O–HOAc (80:20:3). The eluant from the major peak was evaporated to obtain pure **3** (36 mg).

Compound 1: yellow powder; mp 217–218 °C; $[\alpha]^{27}_D$ +162.1 (*c* 0.5, H_2O); UV (H_2O) λ_{max} (log ε) 266 (3.48), 310 (3.52), 392 (3.95) nm; CD (MeOH) 231 ($\Delta\varepsilon$ −11.17), 265 (1.27), 308 (−7.50), 343 (−5.60), 390 (15.40) nm; ^{1}H NMR (D_2O, 600 MHz), Table 1; ^{13}C NMR (150 MHz), Table 1; HR-ESIMS (−ve mode) $[M - H]^-$ at *m*/*z* 465.1035 (calcd for $C_{21}H_{21}O_{12}$, 465.1033).

Compound 2: pale orange powder; mp 141–144 °C; $[\alpha]^{29}_D$ +14.7 (*c* 1.3, MeOH); ^{1}H NMR (D_2O, 300 MHz) δ 7.45 (1H, d, *J* = 9 Hz, H-6′), 6.49 (1H, d, *J* = 9 Hz, H-5′), 4.85 (1H, d, *J* = 8.7 Hz, H-1″), 4.21 (1H, br t, *J* = 8.7 Hz, H-2″), 3.80 (1H, d, *J* = 12.3 Hz, H-6″a), 3.70 (1H, br d, H-6″b), 3.50 (3H, br s, H-3″, H-4″, H-5″); ^{13}C NMR (D_2O, 75 MHz) δ 198.1 (C, C-2), 171.5 (C, C-1), 165.0, 164.4 (both C, C-2′, C-4′), 135.4 (CH, C-6′), 110.0, 108.7 (both C, C-1′, C-3′), 109.7 (CH, C-5′), 80.7 (CH, C-5″), 77.9 (CH, C-3″), 73.4 (CH, C-1″), 70.3 (CH,

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C-2″), 69.8 (CH, C-4″), 60.8 (CH_2, C-6″); HR-ESIMS (−ve mode) $[M - H]^-$ at m/z 343.0676 (calcd for $C_{14}H_{15}O_{10}$, 343.0665).

Compound ***3:*** whitish powder; mp 173−176 °C; $[\alpha]^{29}_D$ +89.3 (c 0.2, MeOH); CD (MeOH) 225 ($\Delta\varepsilon$ 1.94), 257 (−0.56), 291 (2.74), 321 (4.07), 327 (4.12) nm; ^{1}H NMR (methanol-d_4, 300 MHz) δ 7.71 (1H, d, J 8.1 Hz, H-6′), 7.04 (2H, d, J = 7.5 Hz, H-2, H-6), 6.69 (2H, d, J = 7.5 Hz, H-3, H-5), 6.41 (1H, d, J = 7.8 Hz, H-5′), 5.17 (1H, br, H-α), 4.12 (1H, br t, H-2″), 3.88 (1H, d, J = 11.7 Hz, H-6″a), 3.73 (1H, m, H-6″b), 3.47 (3H, m, overlapped by solvent signal, H-3″, 4″, 5″), 3.04 (1H, m, H-βa), 2.85 (1H, m, H-βb), anomeric proton signal not visible due to overlap; ^{13}C NMR (DMSO-d_6, 75 MHz) δ 204.8 (C, CO), 164.5, 164.0 (both C, C-2′, C-4′), 155.7 (C, C-4), 132.0 (CH, C-6′), 130.4 (CH, C-2, C-6), 128.0 (C, C-1), 114.9 (CH, C-3, C-5), 112.3, 110.4 (both C, C-1′, C-3′), 108.2 (CH, C-5′), 81.6 (CH, C-5″), 79.0 (CH, C-3″), 73.2, 72.8, 70.6, 70.4 (all CH, C-1″, C-2″, C-4″, C-α), 61.4 (CH_2, C-6″), 39.8 (CH_2, C-β); HR-ESIMS (+ve mode) $[M + Na]^+$ at m/z 459.1265 (calcd for $C_{21}H_{24}NaO_{10}$ 459.1267).

Preparation of 4 from 1. A solution of **1** (100 mg in 30 mL of H_2O) was treated with 5 mL of 0.1 N NaOH solution. The solution was evaporated; the residue was dissolved in a minimum volume of H_2O and chromatographed over a column of Diaion HP20 set in H_2O. Elution with H_2O afforded compound **4** (46 mg) in the initial fraction.

Compound ***4:*** yellow-orange powder; mp 220−222 °C; UV (H_2O) λ_{max} (log ε) 266 (3.82), 300 (3.84), 394 (4.24), 430 (4.11) nm; CD (MeOH) 208 ($\Delta\varepsilon$ 5.75), 231 (−11.52), 255 (−5.13), 273 (−6.66), 291 (−4.06), 344 (−7.03), 393 (10.01) nm; ^{1}H NMR (D_2O, 600 MHz), Table 1; ^{13}C NMR (D_2O, 150 MHz), Table 1.

Esterification of 1 to 5. A sample of **1** (1.0 g) was suspended in MeOH (7 mL), TFA (2.2 mL) was added, and the mixture was stirred at ambient temperature overnight. Evaporation of the solvent afforded the crude product, which was purified by chromatography over a column of Diaion HP 20 using H_2O followed by increasing percentages of MeOH in H_2O. Compound **5** (0.9 g) was obtained in the 20% MeOH in H_2O eluates.

Compound ***5:*** yellow-orange powder; mp 212−214 °C; $[\alpha]^{27}_D$ +143.3 (c 0.5, MeOH); ^{1}H NMR (methanol-d_4, 600 MHz), Table 1; ^{13}C NMR (methanol-d_4, 150 MHz), Table 1; ESI-MS m/z 503 $[M + Na]^+$.

Acetylation of 5 to 6. Compound **5** (0.4 g) was heated with a mixture of Ac_2O (2 mL) and pyridine (1 mL) at 100 °C for 1 h. Evaporation of the solvent followed by chromatography of the crude product on a column of silica gel afforded the peracetate **6** (0.25 g) in fractions eluted with 30% EtOAc in petroleum ether. The product was obtained solid upon addition of petroleum ether to a benzene solution of the material.

Compound ***6:*** whitish solid; NMR spectra in Supporting Information; ESI-MS m/z 755 $[M + Na]^+$.

Computational Chemistry. A global conformational random search using the MMFF94 molecular mechanics force-field was performed using the SYBYL8.1 program (Tripos International, St. Louis, MO, USA). Density functional theory (DFT) calculations, using Gaussian 03, were employed to optimize the ground-state geometries at 298 K in the gas phase at the B3LYP/6-31G** level. Harmonic frequencies were calculated to confirm the minima. The geometries of the ground states were used to calculate the ECD by using TDDFT at the same level in the gas phase. The calculated excitation energies ΔE_i (in eV) and rotatory strengths (R_i) were simulated into ECD curves by using the Gaussian function

$$\Delta \in (E) = \frac{1}{2.297 \times 10^{-39}} \frac{1}{\sqrt{2\pi}\sigma} \sum_i^E \Delta E_i R_i e^{-[(E-\Delta E_i)/(2\sigma)]^2}$$

where σ is the width of the band at $1/e$ height and ΔE_i and R_i are the excitation energy and rotatory strength for the transition i, respectively. In the current work a value of σ = 0.15 eV and rotatory strength in the dipole length form (R^{len}) were used.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Tables S1−4, Figures S1 and 2, and copies of spectra for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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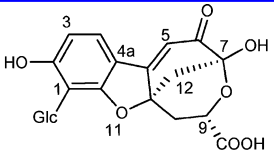
(11) The principal ^{13}C NMR characteristics differentiating the disalt from **1** or the monosalt (carboxylate) (tentatively identified) are the downfield shifts of C-4′ and C-5′ and the upfield shifts of C-5 and C-1′; in the ^{1}H NMR spectrum, the signals of α-H and 2″-H are well resolved (δ 4.41 and 4.16) for **1**, overlapping (at δ 4.31) for **4**, and just resolved (δ 4.10, 4.01) for the monosalt fraction.

(12) The numbering system followed in ref 10 has been used for ease in comparison, but the following systematic nomenclature is preferred for **1** and related compounds.



(7*S*,9*S*,10a*S*)-1-β-D-glucosyl-10,10a-dihydro-2,7-dihydroxy-7,10a-methano-6-oxo-9*H*-8,11-dioxacycloocta[*a*]indene-9-carboxylic acid

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