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Article

Other Chemical Constituents Isolated from *Solanum crinitum* Lam. (Solanaceae)

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O estudo fitoquímico de *Solanum crinitum* Lam forneceu quatro flavonóides: tilirosídeo (**1**), astragalina (**2**), kaempferol (**3**) e biochanina A-7-*O*-β-D-apiofuranosil-(1→5)-β-D-apiofuranosil-(1→6)-β-D-glucopiranosideo (**7**), ácido 4-hidroxibenzoico (**12**), e quatro derivados do ácido cinâmico: *cis*- e *trans*- cumárico (**10** e **11**), *cis*- e *trans*-cumarato de etila (**8** e **9**), isolados de tricomas do fruto. Do extrato metanólico de frutos verdes foram isolados três alcalóides esteroidais glicosilados: solamargina (**13**), 20-epi-solamargina (**14**) e solasonina (**16**). Os derivados 3,5,7,4'-tetra-*O*-metil-kaempferol (**4**), 3,7,4'-tri-*O*-metil-kaempferol (**5**), 3,7,4'-tri-*O*-metil-5-*O*-acetil-kaempferol (**6**), peracetil-*epi*-solamargina (**15**) e peracetil-solasonina (**17**) foram sintetizados e estão sendo registrados pela primeira vez na literatura. As estruturas foram definidas através de análise de dados espectrométricos.

The phytochemical investigation of *Solanum crinitum* Lam led to the isolation from the fruit trichomes of four flavonoids, tiliroside (**1**), astragalin (**2**), kaempferol (**3**), biochanin A-7-*O*-β-D-apiofuranosyl-(1→5)-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside (**7**), along with 4-hydroxybenzoic acid (**12**), and four cinnamic acid derivatives, *cis*- and *trans*-coumaric acids (**10** and **11**) and *cis*- and *trans*- ethyl coumarate (**8** and **9**). Three tri-glycosyl-steroidal alkaloids, solamargine (**13**), 20-*epi*-solamargine (**14**) and solasonine (**16**) were isolated from the methanolic extract of the green fruits. The derivatives 3,5,7,4'-tretra-*O*-methyl-kaempferol (**4**), 3,7,4'-tri-*O*-methyl-kaempferol (**5**), 3,7,4'-tri-*O*-methyl-5-*O*-acetyl-kaempferol (**6**), the peracetyl-*epi*-solamargine (**15**) and peracetyl-solasonine (**17**) were prepared. The structures were established through the analysis of their spectral data. The complete ^{1}H and ^{13}C NMR data assignments of the new peracetyl derivatives of the alkaloids were made.

Keywords: *Solanum crinitum*, Solanaceae, steroidal glycoalkaloids, flavonoids, cinnamic acids

Introduction

Solanum (L) is the most representative genus of Solanaceae, containing about 1,500 species, and 5,000 epithets. It is widespread in tropical and subtropical regions of all the world, but its highest diversity occurs in South America.[1] *Solanum* species are a rich source of steroidal alkaloids, flavonoids and their glycosides which are known to possess a variety of biological activities. The glycoalkaloids are natural toxins with ecological and human health importance, such as the allelopathic effect against herbivores, against pathogenic microorganism[2,3] and molluscicidal activity,[4] and are also of interest as starting material for anabolic, anti-fertility, anti-inflammatory, anti-allergic drugs.[5] As the alkaloids, the flavonoids are a frequent group of compounds in *Solanum* species, and can be used as systematic markers for the family taxons.[6-8]

Solanum crinitum Lam (Solanaceae), popularly known as "jurubeba" and "fruto-de-lobo", is a shrub or small tree in South America, including southern Brazil and Colombia.[9,10] In the course of our pharmacological and phytochemical investigations of *Solanum* species,[3,4,8,11-13] we have reported the cytotoxic and antitumoral activities of flavonoids isolated from trichomes of young branches

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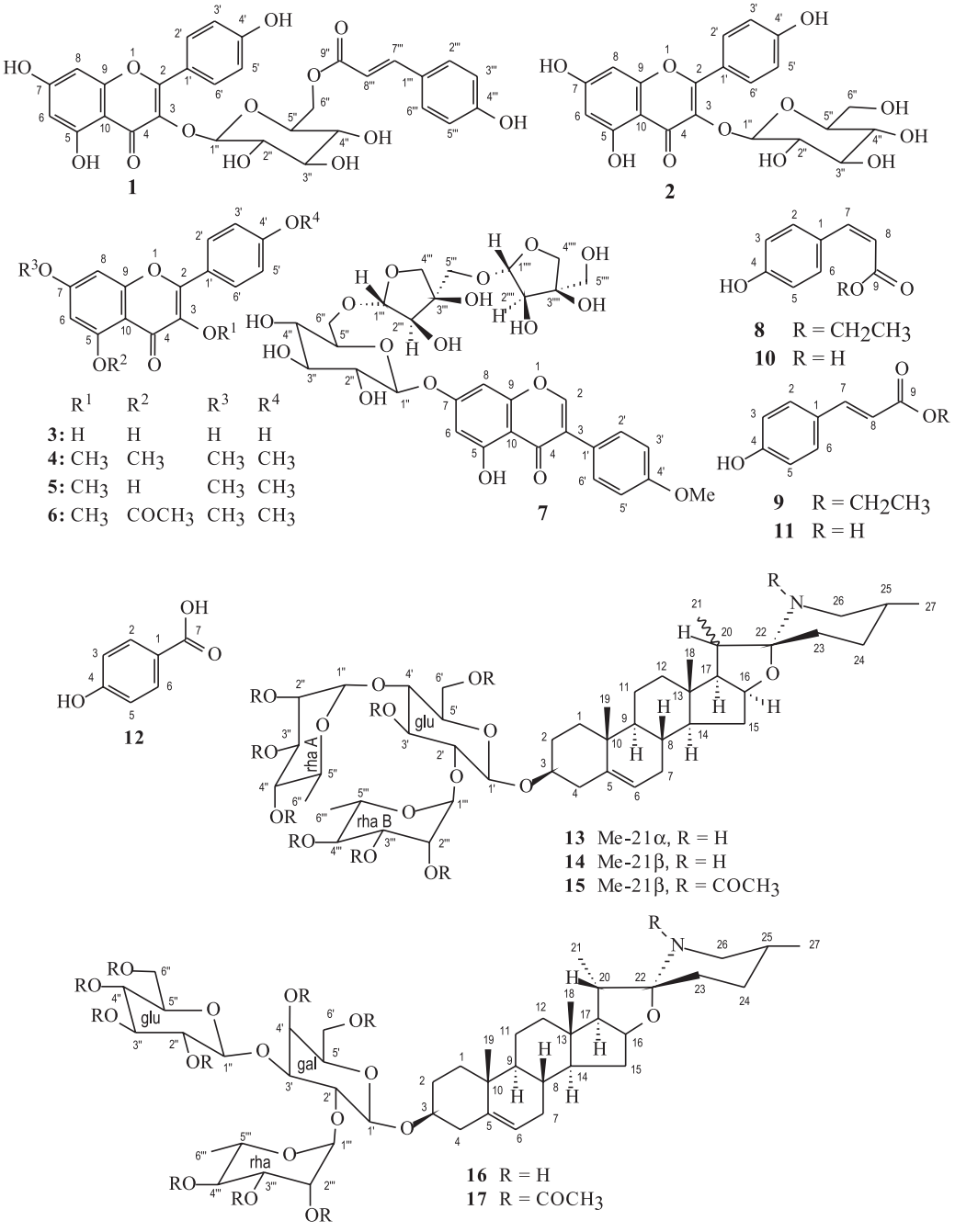
and also of a rich glycoalkaloids total fraction from the green fruits of *Solanum crinitum* Lam[14] along with its allelopathic activity.[3]

This work describes the isolation and structural characterization of the flavonoids tiliroside (**1**), astragalin (**2**), kaempferol (**3**) and biochanin A-7-*O*-β-D-apiofuranosyl-(1→5)-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside (**7**), *cis*- (**10**) and *trans*- (**11**) cumaric acids and ethyl *cis*- (**8**) and *trans*- (**9**) cumarate and 4-hydroxybenzoic acid (**12**), which were isolated from the trichomes, and the glycoalkaloids solamargine (**13**), 20-*epi*-solamargine (**14**) and solasonine (**16**) isolated from the green fruits. The derivatives 3,5,7,4'-tetra-*O*-methyl-kaempferol (**4**), 3,7,4'-tri-*O*-methyl-kaempferol (**5**), 3,7,4'-tri-*O*-methyl-5-*O*-acetyl-kaempferol (**6**), peracetyl-*epi*-solamargine (**15**) and peracetyl-solasonine (**17**) were prepared and used to confirm the structures of the natural compounds. The structures were established through analysis of their spectral data, mainly NMR (1D and 2D) and mass spectra. NMR data for the new acetyl derivatives of the alkaloids are described herein for the first time.



Results and Discussion

From the trichomes isolated from green fruits of *Solanum crinitum* Lam were isolated the flavonoids: tiliroside (**1**), astragalin (**2**), kaempferol (**3**), and biochanin A-7-O-β-D-apiofuranosyl-(1→5)-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside (**7**), *cis*- (**10**) and *trans*- (**11**) cumaric acids and ethyl *cis*- (**8**) and *trans*- (**9**) cumarate esters and 4-hydroxybenzoic acid (**12**); from green fruits methanolic extracts were isolated three tri-glycosyl-steroidal alkaloids, solamargine (**13**), 20-*epi*-solamargine (**14**) and solasonine (**16**). The derivatives 3,5,7,4'-tetramethylkaempferol (**4**), 3,7,4'-trimethylkaempferol (**5**), 3,7,4'-trimethyl-5-acetylkaempferol (**6**), the peracetyl-20-*epi*-solamargine (**15**) and peracetyl-solasonine (**17**) were prepared. The structures were established on the basis of IR, NMR and MS data analysis of the natural compounds and of the derivatives **4**-**6**, **15** and **17**.

Compounds **1**-**3** were identified by the analysis of ^{1}H and ^{13}C NMR spectra, including HMQC and HMBC experiments, and comparison with literature data for tiliroside, kaempferol and astragalin.[15-18] The first report of **1** in *Solanum* species was made by Souza *et al*.[14] Kaempferol and astragalin have been isolated from some *Solanum* species.[8] The treatment of **3** with diazomethane yielded the methyl derivatives **4** and **5**, described in the literature,[19] and the derivative **6** was obtained by treating **5** with Ac_2O/Pyridine. The 2D NMR spectra, including NOESY experiments, of these derivatives were used to confirm the proposed structure of **3** and to carry out the complete assignment of the ^{1}H and ^{13}C chemical shifts of **6** (see Experimental).

The ^{1}H and ^{13}C NMR spectra of compound **7** revealed characteristic resonances of the isoflavonoid biochanin A besides additional signals for three sugars unities, one glucopyranoside and two apiofuranosides. Comparison of these data with those of glycosides isolated from *Dalbergia nigra*[20] and *Andira anthelmia*,[21] besides the analysis of mass spectrum obtained by FAB-MS in positive mode {*m/z* 733.20820 ([M+Na]$^+$, $C_{32}H_{38}O_{18}$+Na$^+$, calc.: *m/z* 733.19558), *m/z* 601.12300 [M+Na-132]$^+$, *m/z* 451.0600 (M+Na-282)$^+$, *m/z* 449.27710 [M+Na-284]$^+$, *m/z* 431.16210 [M+Na-302]$^+$, *m/z* 317.1338 [M+Na-416]$^+$, *m/z* 287.0865 [M+Na-446]$^+$}, led us to identify the compound as biochanin A, 7-*O*-β-D-apiofuranosyl-(1→5)-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside (**7**). This is the first report of this apiofuranosyl derivative in *Solanum* species.

Compounds **8**-**12** were identified by the ^{1}H and ^{13}C NMR spectral data analysis of the mixtures of **8**+**9**, **10**+**11** and of **12** along with comparison with literature data.[21-25] The integration of the ^{1}H NMR signals allowed us to calculate the approximate relative percentage of both compounds: 36.16% of **8** and 63.84% of **9**.

The fractions containing compounds **13**, **14** and **16** showed a positive test for alkaloids. The detailed analysis of the ^{1}H and ^{13}C NMR spectra of the isolated compounds allowed to identify characteristic signals corresponding to the same aglycone as that of the steroidal spirazolane-type alkaloid in the three glycoalkaloids: four quaternary carbon atoms, including one linked to oxygen and nitrogen atoms: (δ_C 98.7 to 98.5 and one sp^2 at δ_C 141.2 to 140.4), nine methine groups (including two oxygenated at δ_C 78.5 to 78.0 and 81.1 to 78.6), ten methylene groups and four methyl groups = $(C)_3(O\text{-}C\text{-}NH)(CH)_7$ $(HC\text{-}O)_2(CH_2)_{10}(CH_3)_4$ = $C_{27}H_{42}NO_3$ = $C_{27}H_{42}NO_2$ considering the presence of an ether function (Table 1) having a trisaccharide moiety (three anomeric carbon atoms: δ_C 100.6 to 100.3) attached to the oxygen atom of carbon CH-3, with δ_C 78.5 to 78.0 (Table 1 and 2), which is significantly higher when compared with the ^{13}C chemical shift of the methyl carbon CH-3 sustaining free hydroxyl group (about δ_C 71). The ^{1}H and ^{13}C chemical shifts of the trisaccharide moieties of **13** and **14**, had practically the same values (Table 2), indicating identical the partial structure *O*-[α-L-rhamnopyranosyl-(1→2)-*O*-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranoside = $C_{18}H_{32}O_{13}$ (three degrees of unsaturation, $C_{18}H_{38}O_{13} - C_{18}H_{32}O_{13} = H_6$). On the other hand, the ^{1}H and ^{13}C signals observed in NMR spectra of **16** allowed to characterize the trisaccharide as *O*-[α-L-rhamnopyranosyl-(1→2)-*O*-[β-glucopyranosyl-(1→3)]-β-D-galactopyranoside = $C_{18}H_{32}O_{14}$ (three degrees of unsaturation, $C_{18}H_{38}O_{14} - C_{18}H_{32}O_{14} = H_6$). All these NMR spectral data allowed to deduce the same molecular formulas $C_{45}H_{73}NO_{15}$ to **13** and **14** and $C_{45}H_{73}NO_{16}$ to **16**, all with ten degrees of unsaturation corresponding to one double bond and six rings). In fact, the values of pseudomolecular peaks in the positive HRMS spectra at *m/z* 868.5239 (M + H$^{\bullet+}$, 100%) of **13** ($C_{45}H_{74}NO_{15}$ = *m/z* 868.5058) and at *m/z* 868.5305 (M + H$^{\bullet+}$, 100%) of **14** ($C_{45}H_{74}NO_{15}$ = *m/z* 868.5058), besides additional peaks compatible with loss of sugar moieties, were used to confirm the molecular formula of these isomeric compounds (**13** and **14**). The hydrogen and carbon atoms signals observed in the ^{1}H and ^{13}C NMR spectra of **13**-**17** (Tables 1 and 3) were also assigned with aid of the homonuclear 2D ^{1}H-^{1}H-COSY and heteronuclear 2D HMQC (^{1}H-^{13}C-COSY-$^1J_{CH}$) and HMBC (^{1}H-^{13}C-COSY-$^nJ_{CH}$, n = 2 and 3), allowing to identify the ^{1}H chemical shifts of the methyl group signals of the aglycone and of the trisaccharide moieties (Table 3 and 4): δ_H 0.83 (d, 3H-27), 0.88 (s, 3H-18), 1.07 (s, 3H-19) and 1.09 (d, 3H-21) of **13**, 0.79 (d, 3H-27), 0.85 (s, 3H-18), 1.06 (s,

3H-19) and 1.28 (d, 3H-21) of **14**, and 0.76 (d, 3H-27), 0.82 (s, 3H-18), 1.03 (s, 3H-19) and 1.17 (d, 3H-21) of **16**; signals of the hydrogen H-6 linked to sp^2 carbons of double bounds at δ_H: 5.32 (**13**), 5.33 (**14**), 5.30 (**16**), besides the signals of H-16 located in the spyrazolane ring (d, *J* 6 Hz) at δ_H 4.42 (**13**); 4.82 (**14**) and 4.21 (**16**). The additional doublets (*J* 6 Hz) corresponding to methyl groups were revealed at δ_{3H} 1.64 and δ_{3H} 1.78 in the spectra of **13** and **14** suggested the presence of two rhamnose moieties in both compounds and only one rhamnose for **16** as indicated by signal at δ_H 1.78. The differences observed in the ^{1}H NMR spectra of **13** and **14**, recorded in same apparatus (^{1}H: 500 MHz; ^{13}C: 125 MHz) and solvent pyridine-d_5, involving mainly the chemical shifts of the hydrogen atoms H-16 (δ_H 4.42 and 4.82), H-17 (δ_H 1.78 and 2.02), H-20 (δ_H 1.98 and 2.14), 2H-26 (δ_H 2.78 and 3.04/2.89) and 3H-21 (δ_H 1.09 and 1.28), which revealed correlation in the HMQC with the ^{13}C signals of the corresponding carbon atoms: CH-16 (δ_C 79.2 and 81.1), CH-17 (δ_C 63.9 and 63.0), CH-20 (δ_C 42.0 and 42.0), CH_2-26 (δ_C 48.2 and 47.1) and 3H-21 (δ_C 16.1 and 15.7), as summarized in Figure 1. Comparative analysis of these data was used to suggest the two stereoisomers H-20β (**13**) and H-20α (**14**), since the 22αN and 22βN possibilities were eliminated considering the absence of ^{13}C signal corresponding to a methylene carbon CH_2-23 at about δ_C 27 which is consistent with the ^{13}C NMR chemical shift of the 22βN stereoisomer (Figure 1). The β-position of the methyl Me-21 located at carbon atom 20 of **14** may be used to justify the ^{13}C chemical shifts of the CH-16 (δ_C 81.1, absence of the γ-effect from Me-21) and CH_2-23 (δ_C 34.0, presence of the γ-effect from Me-21).[26-30] In order to compare the relative stabilities of the epimeric structures **13/14**, a molecular modeling study was implemented using the Spartan 06 for Linux program (Wavefunction, Inc.), see Supplementary Information.[31]

The complete ^{1}H and ^{13}C chemical shift assignments of the signals of CH_3, CH_2, CH and C observed in the ^{1}H (including ^{1}H-^{1}H-COSY) and ^{13}C ({^{1}H} and DEPT) NMR spectra (including 2D experiments HMQC and HMBC) (Tables 1, 2, 3 and 4) and comparison with values described in the literature for solamargine (**13**)[26-29] and solasonine (**16**)[28,31] led to the proposition of the structures (25*R*)(20*S*)(16*S*)-3β-{*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranosyl}-22α*N*-spirosol-5-en (solamargine, **13**), the new glycoalkaloid (25*R*)(20*R*)(16*S*)-3β-{*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranosyl}-22α*N*-spirosol-5-en (16-*epi*-solamargine, **14**), and (25*R*)(20*S*)(16*S*)-3β-{*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-glucopyranosyl-(1→3)]-β-D-galactopyranosyl} -22α*N*-spirosol-5-en (solasonine, **16**) for the three steroidal glycoalkaloids isolated from this plant (Figure 1).

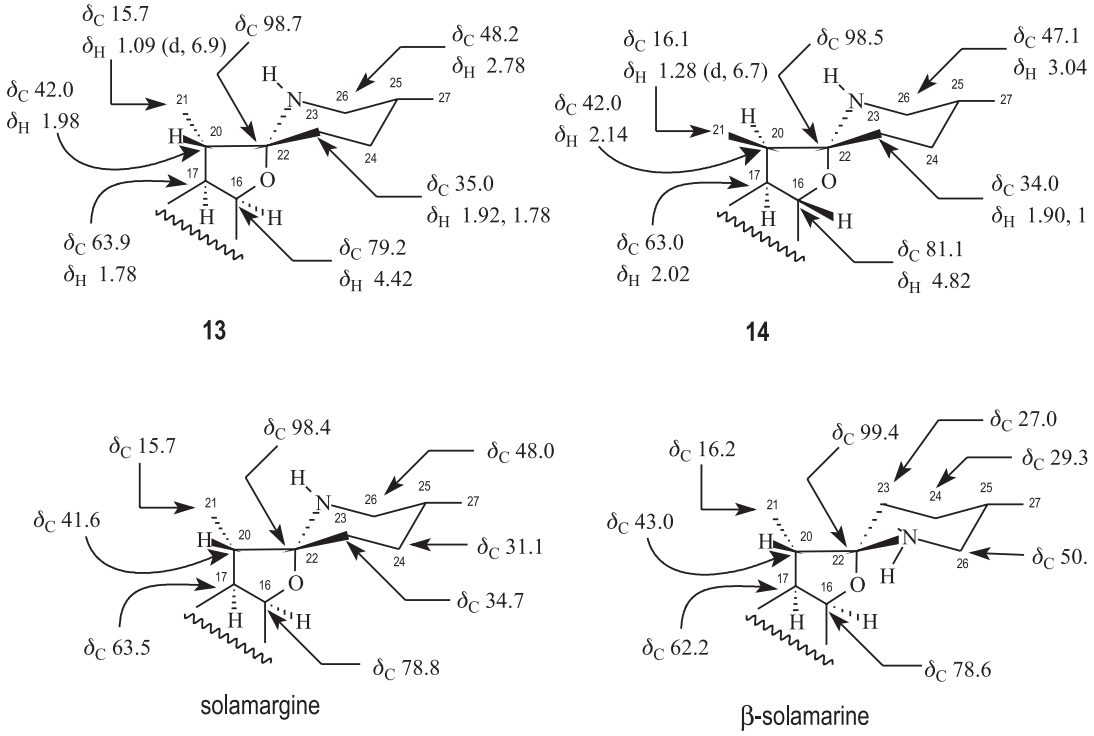


Figure 1. Comparative analysis of the chemical shifts corresponding to the hydrogen (δ_H) and carbon (δ_C) atoms CH-16, CH-17, CH-20, CH_3-21, C-22, CH_2-23 and CH_2-26 of **13** and **14**, including comparison of the δ_C these carbon atoms and of the CH_2-24 to reveal the structural distinction between solamargine and β-solamarine.

Table 1. ^{13}C NMR spectral data for aglycone of **13**, **14** and **16** in pyridine-d_5 (125 MHz: **13** and **14**; 50 MHz: **16**) and of peracetyl derivatives **15** and **17** in $CDCl_3$ (125 MHz)*

C	13	14	15[a]	16	17[a]
1 (CH_2)	37.9	37.5	37.3	37.6	37.1
2 (CH_2)	30.5	30.2	29.7	30.2	29.5
3 (CH)	78.5	78.0	79.3	78.4	79.8
4 (CH_2)	39.3	39.0	38.4	38.8	38.4
5 (C)	141.2	140.4	140.4	141.0	140.2
6 (CH)	122.2	121.9	122.2	121.8	121.9
7 (CH_2)	32.1	32.3	31.8	32.3	31.6
8 (CH)	32.0	31.7	31.6	31.8	31.4
9 (CH)	50.7	50.3	50.3	50.3	50.0
10 (C)	37.5	37.2	36.9	37.2	36.7
11 (CH_2)	21.5	21.1	21.0	21.2	20.6
12 (CH_2)	40.5	39.7	40.0	39.9	39.8
13 (C)	41.0	40.8	42.9	40.8	42.7
14 (CH)	57.0	56.5	53.0	56.6	54.8
15 (CH_2)	32.9	32.6	31.6	32.6	31.6
16 (CH)	79.2	81.1	75.9	78.6	75.6
17 (CH)	63.9	63.0	62.1	63.2	62.0
18 (CH_3)	16.9	16.4	12.8	16.5	12.4
19 (CH_3)	19.8	19.4	19.4	19.5	20.3
20 (CH)	42.0	42.0	40.0	41.9	39.7
21 (CH_3)	16.1	15.7	21.1	15.8	21.4
22 (C)	98.7	98.5	99.7	98.5	99.2
23 (CH_2)	35.0	34.0	35.2	34.3	35.0
24 (CH_2)	31.4	30.0	30.1	30.5	30.8
25 (CH)	32.1	31.7	29.1	30.8	28.9
26 (CH_2)	48.2	47.1	45.5	47.5	45.2
27 (CH_3)	20.1	19.2	18.5	19.5	19.2

* Number of hydrogens bound to carbon atoms deduced by comparative analysis of {^1H}- and DEPT-^{13}C NMR spectra. 2D ^1H-^1H-COSY and ^1H-^{13}C-$^nJ_{CH}$ (n = 2 and 3) NMR spectra were also used in these assignments; [a] δ_{COCH_3}: 170.1-170.2 (CO), 23.8 (CH_3).

The natural alkaloids **14** and **16** were treated with Ac_2O/pyridine to yield the peracetyl derivatives **15** and **17**. The ^1H and ^{13}C NMR spectral data of these peracetyl derivatives (**15** and **17**), obtained through the analysis of extensive 1D and 2D NMR experiments (Tables 1-4), were also used to confirm the postulated structures to **14** and **16**. The analysis of 1D and 2D NMR spectra was also used to make the complete hydrogen and carbon-13 chemical shift assignments for the alkaloid, the new 16-*epi*-solamargine (**14**) and for the two peracetyl derivatives **15** and **17**.

Experimental

General procedure

Melting points have not been corrected. IR, NMR and mass spectra were recorded on the same equipments used in previous papers.[21,32] Column chromatography was carried out with silica gel (Vetec and Aldrich 0.05-0.20 mm) and Sephadex LH-20 (Sigma, USA); silica gel F254 G (Vetec) was used for preparative TLC; aluminum backed (Sorbent) silica gel plates W/UV254 were used for analytical TLC, with visualization under UV (254 and 366 nm), with $AlCl_3$-ETOH (1%), Liebermann-Burchard and/or Godin reagents, or exposure to iodine vapor.

Plant material

The green fruits of *Solanum crinitum* Lam. were collected in September, 2001, in the campus of Universidade Federal Rural do Rio de Janeiro (UFRRJ), Seropédica-RJ, Brazil. They were collected by M.Sc. José Milton Alves (Agronomy Institute, UFRRJ). The identification was made by Dr. Maria de Fátima Agra, Laboratório de Tecnologia Farmacêutica, Universidade Federal da Paraíba, João Pessoa-PB. A voucher specimen (No. JP-28000) was deposited at the Herbarium Prof. Lauro Pires Xavier, Universidade Federal da Paraíba, João Pessoa-PB, Brazil.

Extraction and isolation

The trichomes (9.7 g) were isolated by scraping the green fruits with a glass slide and were subsequently extracted with $CHCl_3$ and MeOH in an ultrasound bath to furnish the $CHCl_3$ extract (310.0 mg) and MeOH (3.0 g) residues. The $CHCl_3$ residue was chromatographed on a sephadex CC, using MeOH as eluent, and 13 fractions were collected and analyzed by TLC and ^1H NMR spectroscopy. Fractions 7-8 yielded astragaline (**1**, 10.0 mg) and fractions 9-10 yielded the tilirozide (**2**, 25.0 mg). The methanolic extract was chromatographed on a silica gel column (col A) and 20 fractions were collected and analyzed by TLC plate. Fractions 5-8 (2.0 g) were dissolved in methanol and addition of CH_2Cl_2 yielded a precipitate that was separated by filtration to afford a solid (1.6 g) that was identified as kaempferol (**3**, mp 282-284 °C) and the mother liquor (AM-5-8). The reaction of kaempferol (65.0 mg) with diazomethane yielded 16.1 mg of **4** (mp 128-130 °C) and 36.6 mg of **5** (mp 140-142 °C) that were separated by preparative TLC. 20.3 mg of **5** were treated with Ac_2O/pyridine (1:1) to afford **6** (14.8 mg, mp 166-168 °C). The mother liquor (AM 5-8, 360.0 mg) was submitted to silica

Table 2. ^{13}C NMR spectral data for glycoside moieties of **13**, **14** and **16** in pyridine-d_5 (125 MHz: **13** and **14**; 50 MHz: **16**) and of peracetyl derivatives **15** and **17** in $CDCl_3$ (125 MHz)*

C	trisaccharide of **13**	trisaccharide of **14**	trisaccharide of **15**[a]	trisaccharide of **16**	trisaccharide of **17**[a]
1' (CH)	100.6	100.3	99.8	100.4	99.6
2' (CH)	78.2	77.8	76.5	76.6	73.1
3' (CH)	78.3	78.1	75.5	84.9	78.3
4' (CH)	79.0	78.8	77.9	70.5	69.5
5' (CH)	77.3	76.9	72.5	77.5	70.9
6' (CH_2)	61.7	61.3	62.1	62.6	62.0
1" (CH)	103.3	102.9	99.7	105.9	99.2
2" (CH)	72.9	72.5	70.5	72.6	71.8
3" (CH)	73.1	72.8	68.8	80.2	72.3
4" (CH)	74.3	73.9	70.8	75.2	68.2
5" (CH)	70.8	70.5	68.1	77.5	72.0
6" (CH_3)	18.9	18.5	17.4	62.6	60.9
1"' (CH)	102.4	102.1	97.5	102.3	97.0
2"' (CH)	72.9	72.6	70.2	72.9	68.9
3"' (CH)	73.1	72.9	68.8	74.2	69.1
4"' (CH)	74.5	74.2	71.5	75.0	70.9
5"' (CH)	69.9	69.5	66.7	69.5	66.5
6"' (CH_3)	19.0	18.7	17.5	18.7	17.1

* Number of hydrogens bound to carbon atoms deduced by comparative analysis of {^1H}- and DEPT-^{13}C NMR spectra. 2D ^1H-^1H-COSY and ^1H-^{13}C-$^nJ_{CH}$ (n = 2 and 3) NMR spectra were also used in these assignments; [a] δ_{COCH_3}: 169.9-170.9 (CO), 20.9-21.7 (CH_3).

gel CC and fractions 8-12 yielded a mixture of **8** and **9** (1.2 mg, gum). Fraction 10 (850 mg) from col A was chromatographed on a silica gel column and 130 fractions of 50 mL were collected. Fractions 32-40 yielded 35.5 mg of a material corresponding to the mixture of **10**, **11** and **12**. Fractions 94-114 (102.3 mg) were chromatographed on a silica gel column and fractions 3-5 yielded **7** (9.5 mg) after filtration on Sephadex LH20.

The green fruits (2.6 g) of *Solanum crinitum* Lam were powdered and extracted with ethanol + acetic acid (2%) and 900 mL of solution was obtained. 900 mL of acetic acid (10%) were added, and the solution was left to stand and chill overnight. The solution was filtered under vacuum using Hirsch funnel. NH_4OH (pH 9-10) was added to the filtrate which was allowed to stand overnight in the fridge affording a precipitate (96.4 g) corresponding to the glycoalkaloids fraction was obtained. 90 g of the glycoalkaloids was adsorbed on silica gel and applied on a silica gel column, eluted with hexane, dichloromethane, ethyl acetate, acetone and methanol. 14 fractions of 500 mL were collected. Fraction 8 (1.0 g), collected with ethyl acetate, was chromatographed in a silica gel CC and fractions analyzed by silica gel TLC plate. Fraction 84 (52.6 mg) from this column was filtered on a Sephadex LH20 column, eluted with methanol, and the alkaloid solamargine **13** (268-270 °C) was obtained. Fraction 10 (8.67 g) was extracted with methanol and the alkaloids were detected with Dragendorff and Libermann Burchard. This fraction was chromatographed on a silica gel column using CH_2Cl_2/MeOH (3:1) as initial eluent. 79 fractions were collected and were analyzed by TLC plate and reunited in group. Fractions 24-29 (1.2 g) were filtered on a Sephadex LH-20 column and the *epi*-solamargine (**14**, 225.8 mg, 238-250 °C) was obtained. Acetic anhydride:pyridine (1:1) treatment of **14** (44.5 mg) yielded 44.5 mg of the peracetyl derivative **15** (mp 128-130 °C). Fractions 39-44 (1.3 g) were filtered on Sephadex LH20 to isolate solasonine (**16**, 93.5 mg, mp 244-246 °C). Treatment of **16** (79.1 mg) with acetic anhydride:pyridine (1:1) gave the peracetyl-solasonine (**17**, 27.6 mg, mp 148-150 °C).

*3,7,4'-trimethoxy-5-acethoxyflavone (**6**)*

Yellow powder, mp 166-168 °C; IR (KBr) ν_{max}/cm^{-1}: 1762, 1632, 1606, 1511; ^1H NMR ($CDCl_3$, 500 MHz) δ 6.60 (d, *J* 2.5 Hz), 6.82 (d, *J* 2.5 Hz), 8.02 (dd, *J* 9.0 Hz), 7.02 (d, *J* 9.0 Hz), 3.78, 3.90 (s), 3.89 (s), 2.47 (s), ^{13}C NMR ($CDCl_3$, 125 MHz) δ 154.8 (C-2), 140.8 (C-3), 173.2 (C-4), 150.5 (C-5), 108.0 (CH-6), 163.1 (C-7), 98.6 (CH-8), 157.8 (C-9), 111.4 (C-10), 122.9 (C-1'), 129.9 (CH-2',6'), 114.0 (CH-3',5'), 161.4 (C-4'), 59.9, 55.9, 55.3 (MeO-3,7,4', respectively), 169.7/21.2 ($COCH_3$-5).

Table 3. ^{1}H NMR spectral data for aglycone of **13**, **14** and **16** in pyridine-d_5 (500 MHz: **13** and **14**; 200 MHz: **16**) and of peracetyl derivatives **15** and **17** in $CDCl_3$ (500 MHz). Chemical shifts in δ (ppm) and coupling constants (*J* in parentheses) in Hz*

H	13	14	15[a]	16	17[a]
1	1.73/1.00	1.75/1.02	1.85/1.08		1.85/1.08
2	2.10/1.87	2.08/1.85	1.93/1.58		1.95/1.25
3	3.89 (m)	3.89 (m)	3.60	3.93 (m)	3.58 (m)
4	2.82/2.75	2.81 (dd, *J* 11.6; 3.1) 2.73 (t, *J* 11.6)	2.40/2.25	2.87/2.76	2.45/2.28
6	5.32 (br s)	5.33 (br s)	5.36 (m)	5.30 (br s)	5.38 (m)
7	1.90	1.85	1.94		2.30/1.68
8	1.48	1.58	1.56		1.53
9	0.90	0.89(m)	0.95		0.95
11	1.45	1.42	1.55-1.45		1.55-1.45
12	1.63/1.05	1.68/1.09	2.08/1.25		2.08/1.25
14	1.10	1.09 (m)	0.98		0.98
15	2.08/1.45	2.08/1.48	2.20/1.70		1.95/1.85
16	4.42	4.82	5.06	4.21	5.08
17	1.78	2.02	1.53		1.53
18	0.88 (s)	0.85 (s)	0.87 (s)	0.82 (s)	0.91 (s)
19	1.07 (s)	1.06 (s)	1.01 (s)	1.03 (s)	1.04 (s)
20	1.98	2.14	2.10		2.08
21	1.09 (d, *J* 6.9)	1.28 (d, *J* 6.7)	1.26 (d, *J* 6.9)	1.17 (d)	1.26 (d, *J* 7.0)
23	1.90/1.65	1.92/1.78	2.36/1.04		2.35/1.03
24	1.52	1.65	2.28/1.68		1.70/1.65
25	1.65	1.58	2.02		2.02
26	2.78	3.04 (m)/2.89 (t, *J* 11.5)	3.20	2.83	3.16(m)
27	0.83 (d, *J* 4.8)	0.79 (d, *J* 6.5)	0.92 (d, *J* 6.6)	0.76(d)	0.93 (d, *J* 7.5)

*Number of hydrogens bound to carbon atoms deduced by comparative analysis of {^{1}H}- and DEPT-^{13}C NMR spectra. Chemical shifts and coupling constants (*J*, in parentheses) obtained from 1D ^{1}H NMR spectra. 2D ^{1}H-^{1}H-COSY and ^{1}H-^{13}C-$^nJ_{CH}$ (n = 2 and 3) NMR spectra were also used in these assignments; [a] $\delta_{COC\underline{H}_3}$: 2.16 (s).

*Epi-solamargine (**14**)*

Colorless crystals, mp 238-250 °C; $[\alpha]^{25}_D$ – 98 (*c* 0.1, MeOH); IR (KBr) ν_{max}/cm^{-1}: 3417, 2927, 1453, 1384, 1044 cm^{-1}. HR-FABMS positive ions at *m/z* 868.5239 $[M+H]^+$ (calc. for $C_{45}H_{74}NO_{15}$: *m/z* 868.505846), 722.4643 (calc. for $C_{39}H_{64}NO_{11}$: 722.447937) $[M+H\text{-rhamnose}]^+$, *m/z* 576.4037 (calc. for $C_{33}H_{54}NO_7$: 576.390028) $[M+H\text{-rhamnose-rhamnose}]^+$, *m/z* 445.7553 (calc. for $C_{21}H_{33}O_{10}$: 445.207372) $[M-423]^+$, *m/z* 414.3467 (calc. for $C_{27}H_{44}NO_2$: 414.337204) $[M+H\text{-rhamnose-rhamnose-glucose}]^+$, *m/z* 413.2782 (calc. for $C_{27}H_{43}NO_2$: 413.329379) $[M\text{-rhamnose-rhamnose-glucose}]^+$. ^{13}C RMN: Tables 1 and 2. ^{1}H RMN: Tables 3 and 4.

*Peracetyl-epi-solamargine (**15**)*

Crystal, mp 128-130 °C, $[\alpha]^{25}_D$ – 165 (*c* 0.1, MeOH); IR (KBr) ν_{max}/cm^{-1}: 2943. 2728, 1751, 1438, 1374, 1045; Positive ion FABMS *m/z* 1246.5522 $[M+H]^+$ (calc. for $C_{63}H_{92}NO_{24}$: 1246.600928), *m/z* 1204.5278 (calc. for $C_{61}H_{90}NO_{23}$: 1204.590364) $[M+H\text{-}42]^+$, *m/z* 974.5256 (calc. for $C_{51}H_{76}NO_{17}$: 974.511325) $[M+H\text{-rhamnose}]^+$, *m/z* 932.5163 (calc. for $C_{49}H_{74}NO_{16}$: 932.500760) $[M\text{-}314]^+$, *m/z* 634.7963 (calc. for $C_{39}H_{56}NO_6$: 634.410763) $[M+H\text{-}612]^+$, *m/z* 413.2715 (calc. for $C_{27}H_{43}NO_2$: 413.329379) [M-acetate-rhamnose-rhamnose-glucose]$^+$. ^{13}C RMN: Tables 1 and 2. ^{1}H RMN: Tables 3 and 4.

*Peracetyl-solasonine (**17**)*

Crystal, mp 148-150 °C; ^{13}C RMN: Tables 1 and 2; ^{1}H RMN: Tables 3 and 4.

Supplementary Information

Supplementary data associated with this paper are available free of charge at http://jbcs.sbq.org.br, as a PDF file including a molecular modeling study in order to

Table 4. ^{1}H NMR spectral data for glycoside moieties of **13**, **14** and **16** in pyridine-d_5 (500 MHz: **13** and **14**; 200 MHz: **16**) and of peracetyl derivatives **15** and **17** in $CDCl_3$ (500 MHz). Chemical shifts in δ (ppm) and coupling constants (*J* in parentheses) in Hz*

H	Glycoside unit of **13**	Glycoside unit of **14**	Glycoside unit of **15**[a]	Glycoside unit of **16**	Glycoside unit of **17**[a]
1'	4.95	4.96	4.56 (d, *J* 7.7)	4.95	4.46 (d, *J* 8.0)
2'	4.24	4.22	3.56	3.98	3.84 (dd, *J* 8.0; 9.5)
3'	4.23 (t)	4.23	5.28 (t, *J* 9.4)	4.33 (m)	3.91 (dd, *J* 9.5; 3.0)
4'	4.42 (t)	4.40	3.73 (t, *J* 9.4)	4.82	5.26 (br s)
5'	3.65 (br d, *J* 9.2)	3.66 (td, *J* 9.2)	3.59 (m)		3.79 (m)
6'	4.22(br d, *J* 12.0) 4.10 (br d, *J* 12.0)	4.23 (dd, *J* 12.1; 3.2)n 4.10 (dd, *J* 12.1; 3.2)	4.44 (d, *J* 12.3) 4.28 (dd, *J* 12.3; 3.6)		4.12 (m) 4.06 (m)
1"	5.86 (s)	5.87 (s)	4.79 (d, *J* 1.5)	5.17(d)	4.71 (d, *J* 8.0)
2"	4.69 (sl)	4.69(d, *J* 1.4)	5.02 (m)	4.68 (sl)	4.85 (dd)
3"	4.55 (dd, *J* 9.1; 2.9)	4.55 (dd, *J* 9.2; 3.2)	5.16 (dd, *J* 10.2; 3.1)		5.22 (t)
4"	4.38	4.34 (t, *J* 9.2)	5.03 (t, *J* 10.2)		5.08
5"	4.92	4.93 (m)	3.85 (m)		3.69 (m)
6"	1.64 (d, *J* 5.9)	1.64 (d, *J* 6.1)	1.14 (d, *J* 6.1)		4.38 (br d, *J* 10.5)/4.10
1"'	6.41 (s)	6.41 (s)	4.90 (d, *J* 1.5)	6.28 (s)	5.02 (sl)
2"'	4.84 (br s)	4.84 (d, *J* 1.8)	5.00 (m)	4.90 (d)	5.22
3"'	4.64 (dd, *J* 9.1; 2.9	4.64 (dd, *J* 9.2; 3.3)	5.23 (dd, *J* 10.1 Hz; 3.1)	4.61	5.22
4"'	4.34	4.32 (t, *J* 9.2)	5.05 (t, *J* 10.1)		5.02(m)
5"'	4.96	4.98 (m)	4.36 (m)	4.88 (br s)	4.43 (m)
6"'	1.78 (d, *J* 6.0)	1.78 (d, *J* 6.2)	1.17 (d, *J* 6.2)	1.78 (d)	1.19 (d, *J* 6.0)

*Number of hydrogen bound to carbon atoms deduced by comparative analysis of {^{1}H}- and DEPT-^{13}C NMR spectra. Chemical shifts and coupling constants (*J*, in parentheses) obtained from 1D ^{1}H NMR spectra. 2D ^{1}H-^{1}H-COSY and ^{1}H-^{13}C-$^{n}J_{CH}$ (n = 2 and 3) NMR spectra were also used in these assignments; [a] δ_{COCH_3}: 1.98-2.15 (8×CH_3).

compare the relative stabilities of the epimeric structures **13** and **14**.

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Other Chemical Constituents Isolated from *Solanum crinitum* Lam. (Solanaceae)

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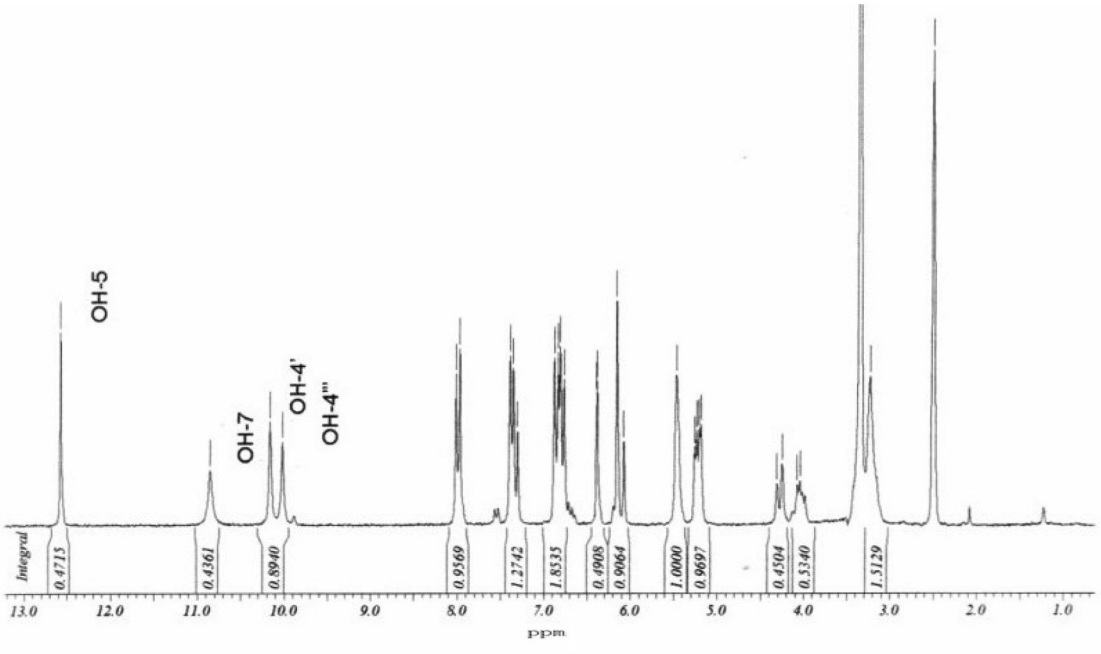

Figure S1. ^{1}H NMR spectrum of compound **1**.

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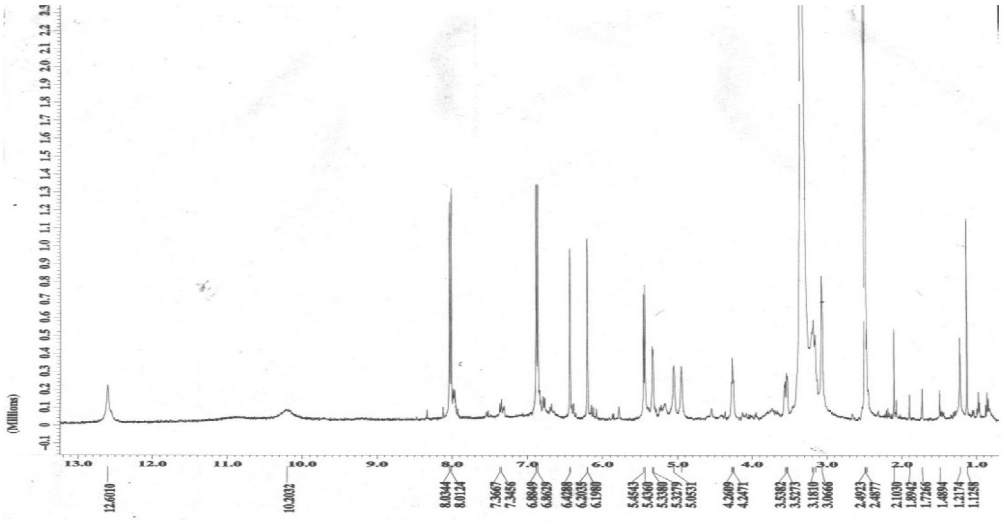

Figure S2. ^{1}H NMR spectrum of compound **2**.

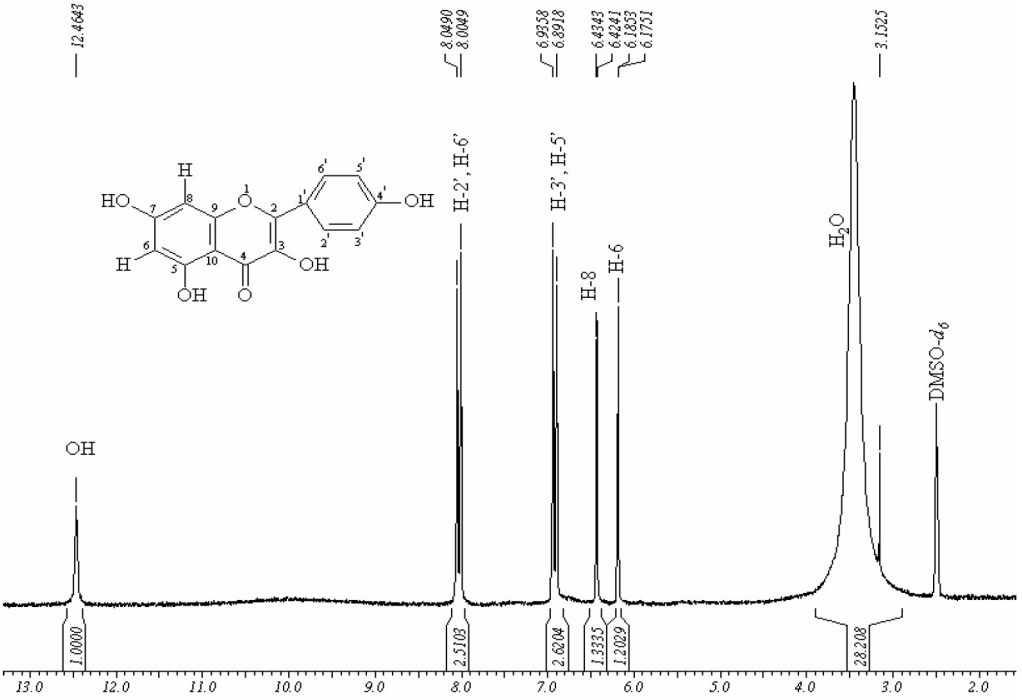

Figure S3. ^{1}H NMR spectrum of compound **3**.

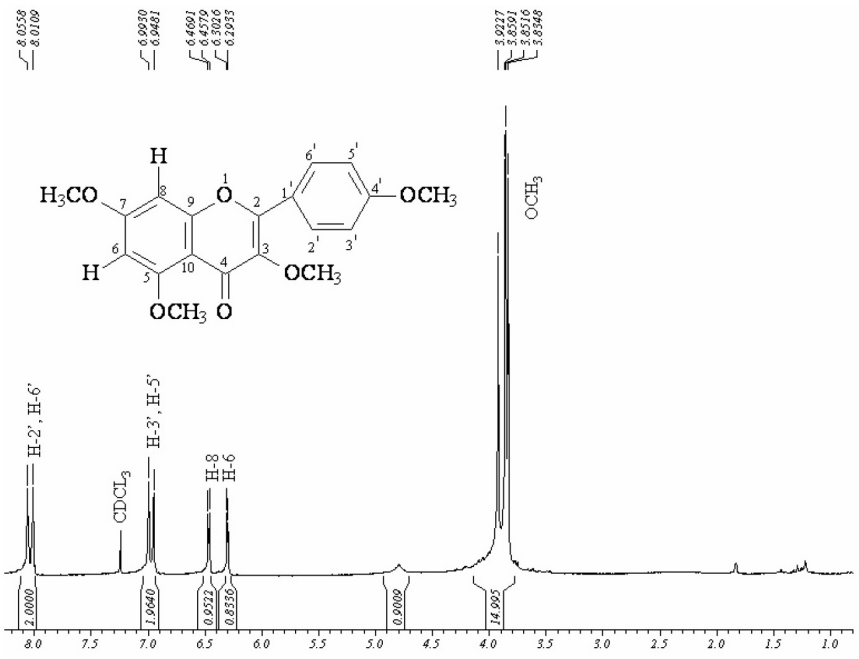

Figure S4. ^{13}C NMR spectrum of compound **4**.

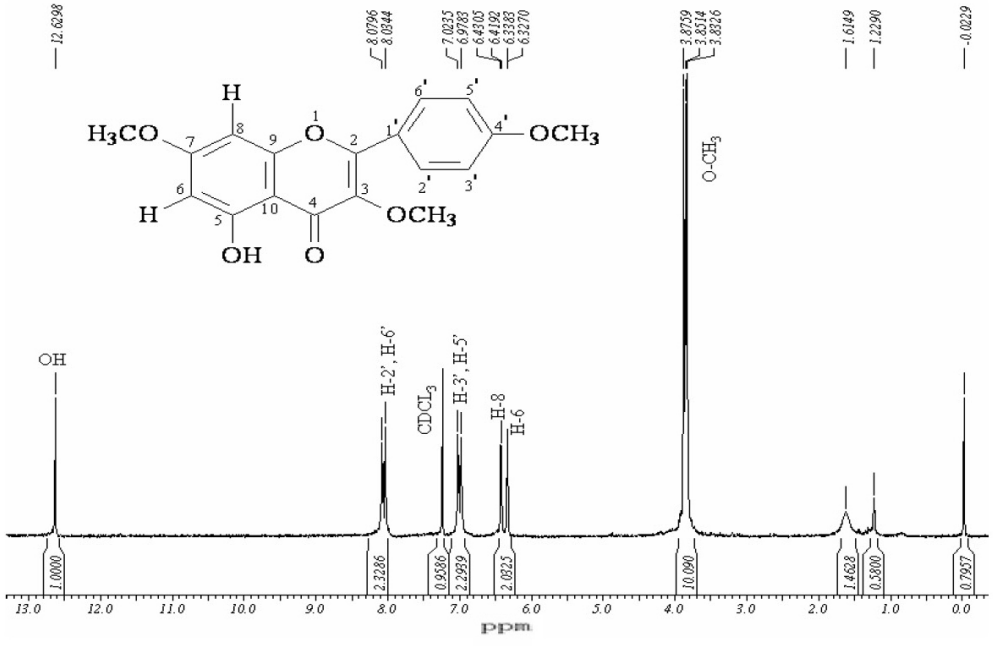

Figure S5. ^{1}H NMR spectrum of compound **5**.

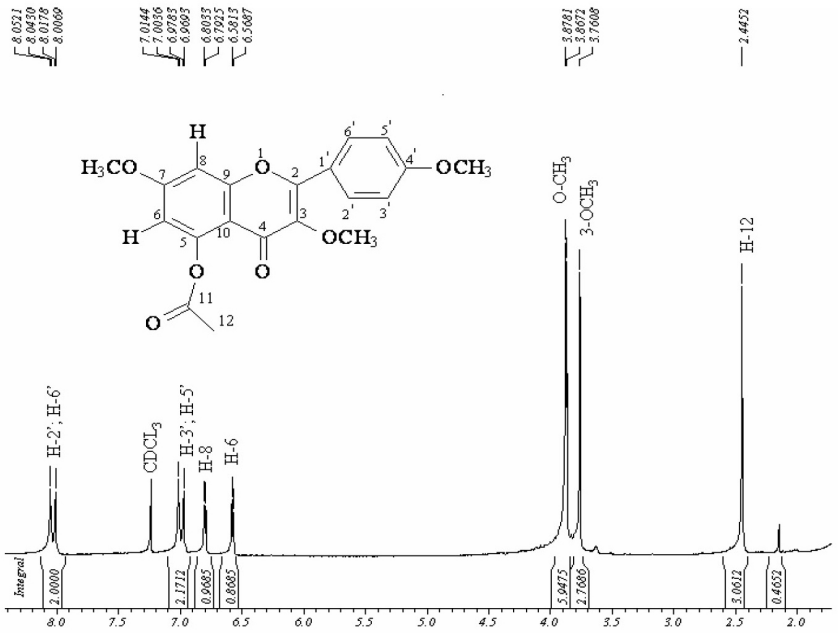

Figure S6. [1]H NMR spectrum of compound **6**.

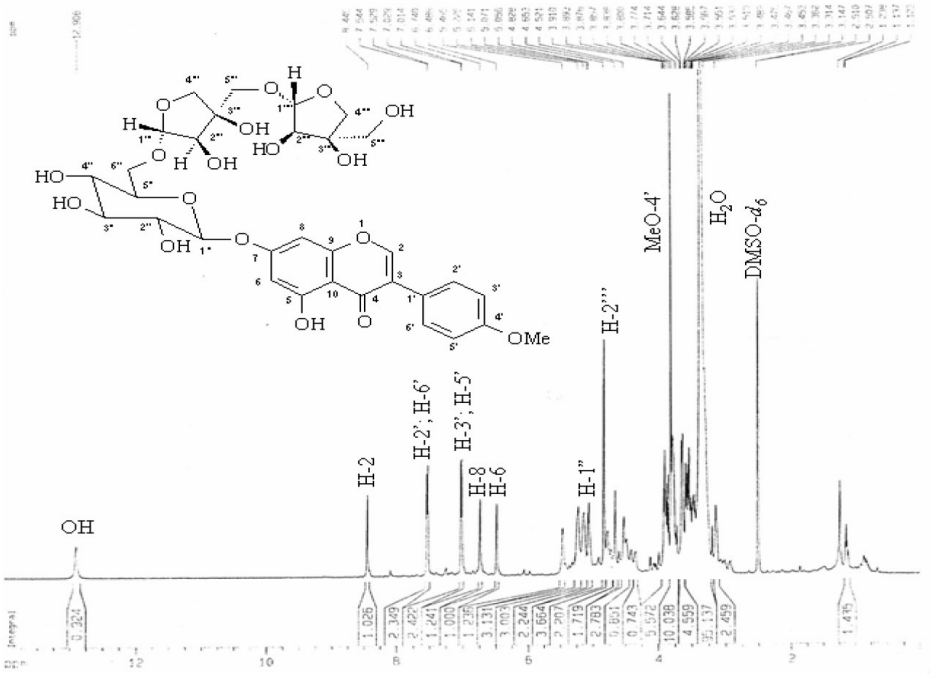

Figure S7. [1]H NMR (500 MHz) of compound **7**.

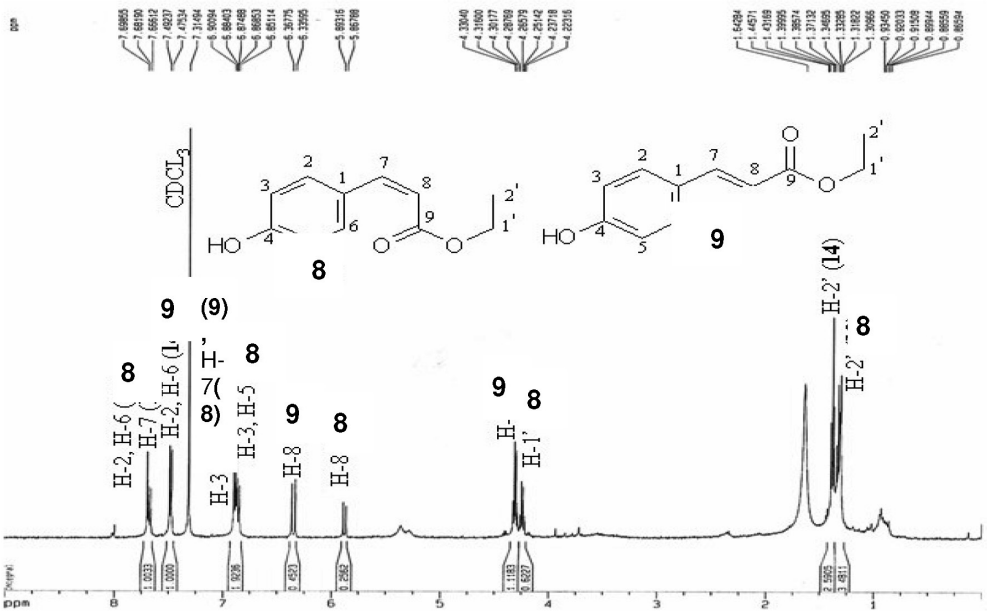

Figure S8. [1]H NMR spectrum(500 MHz) of compounds **8**+**9**.

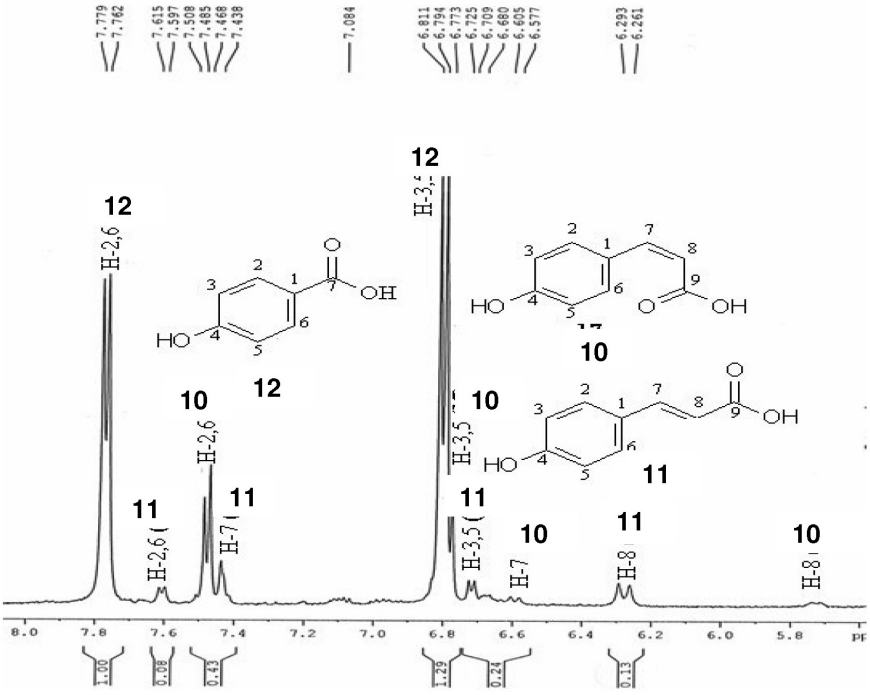

Figure S9. [1]H NMR spectrum (500 MHz) of compounds **10**+**11**+**12**.

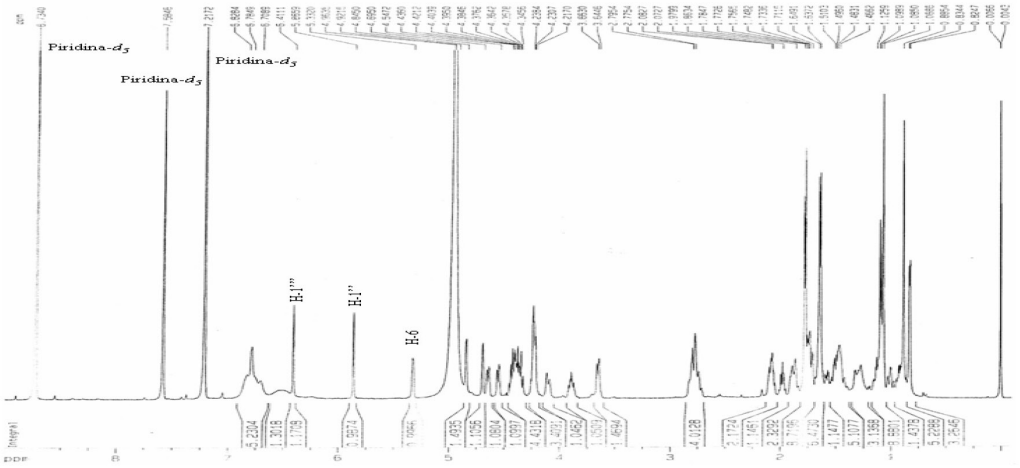

Figure S10. ^{1}H NMR spectrum (500 MHz, Pyridine-d_5) of the compound **13**.

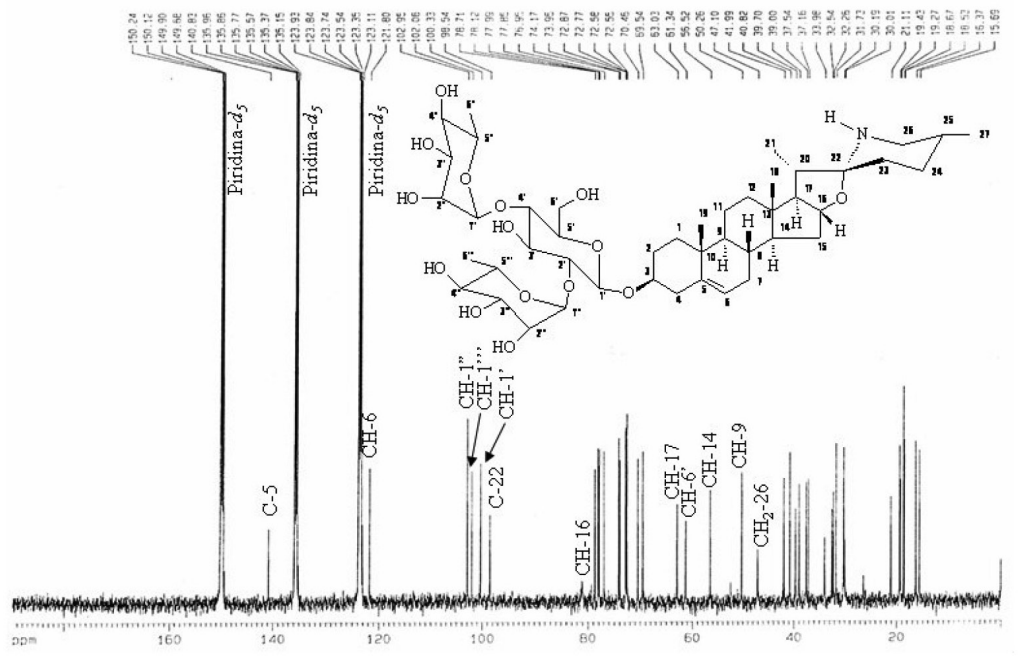

Figure S11. ^{13}C NMR (125 MHz) of compound **14**.

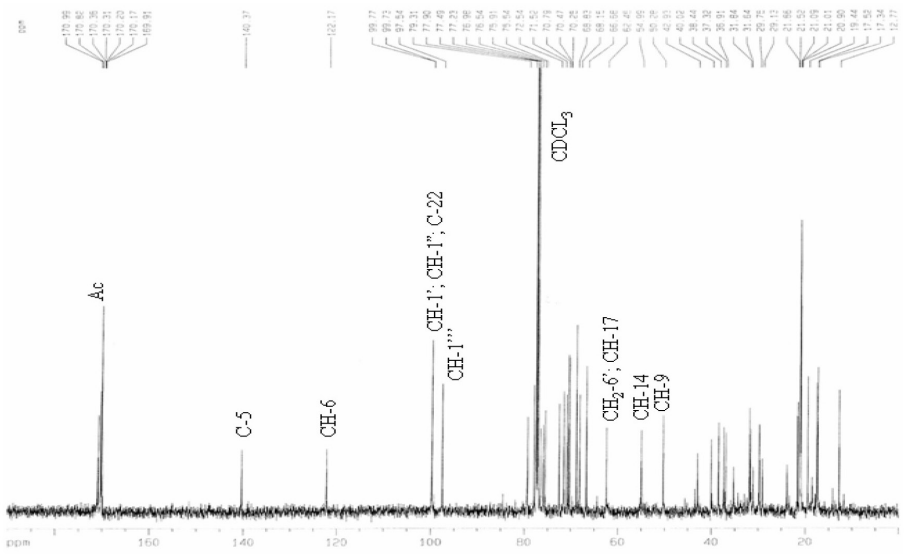

Figure S12. ^{13}C NMR spectrum (125 MHz) of compound **15.**

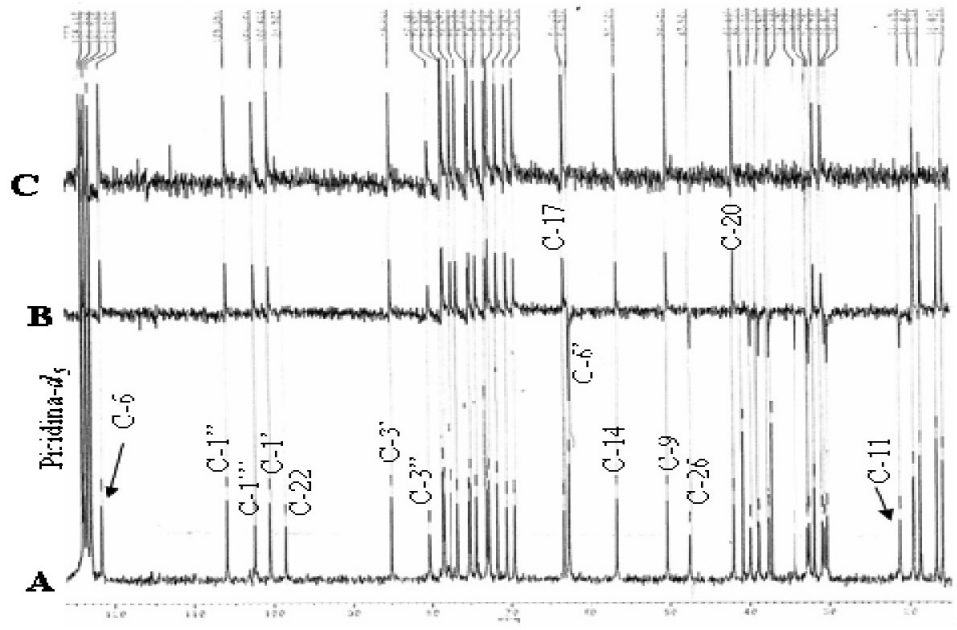

Figure S13. ^{13}C NMR spectrum BBD (A) and DEPT-135 ((B and C), 50 MHz, pyridine-d_6) of **16**.

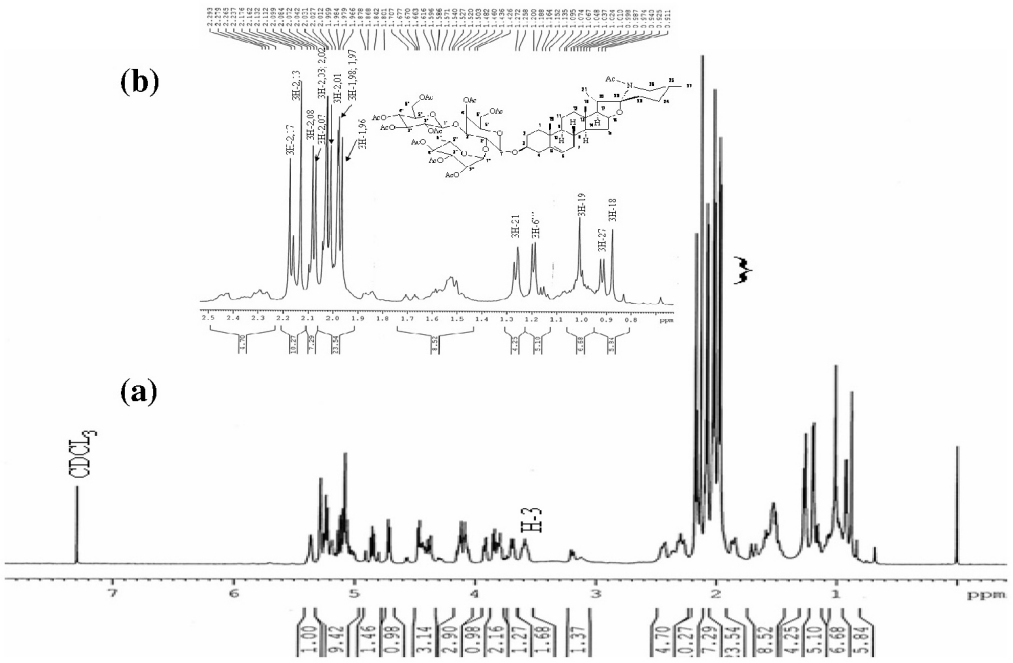

Figure S14. ^{1}H NMR spectrum (a) and expansion (b) (400 MHz, $CDCl_3$) of **17**.

Molecular Modeling

In order to compare the relative stabilities of the epimeric structures **13/14**, a molecular modeling study was implemented using the Spartan 06 for Linux program (Wavefunction, Inc.). Because the long chain attached to C-3 should only have a small influence on the relative stabilities, it was replaced by a methoxy group to reduce the computational cost for the calculations. The conformer distribution of the resulting alpha and beta epimer models (**13a** and **14a**) was determined with the Monte Carlo approach using the MMFF molecular mechanics force field. The most stable conformers of **13a** and **14a** were submitted to a previous energy minimization with the PM3 semi empirical method.[31] The PM3 optimized structures were then submitted to a complete energy minimization with the B3LYP/6-31G* DFT method. The B3LYP method was chosen because it usually yields results for many properties in close agreement with those obtained from MP calculations, and is more efficient than conventional *ab initio* correlated methods for larger-scale calculations. The possibility of existence of this epimer as a stable species was verified by a molecular modeling study at the DFT B3LYP/6-31G* level with models of both epimers. The β-epimer model at C-20 (**14a**) is less stable than the α-epimer one (**13a**), but the energy difference between both structures is only 4.85 kcal mol^{-1} (20.30 kJ mol^{-1}), as calculated with the B3LYP/6-31G* DFT method. Because of this small energy difference, the β-epimer is expected to exist in appreciable amount in an equilibrium mixture with the more stable α-epimer. The main reason for the lower stability of the β-epimer should be the proximity between the C-21 methyl group and carbons C-23 and C-18. The corresponding C-C distances, which are equal to 4.22 Å and 3.47 Å, respectively, in **13a**, are considerably shorter in **14a**, 3.43 Å and 2.98 Å, respectively. This closer proximity would raise more unfavorable steric interactions in **14a** than in **13a** (Figure S15).

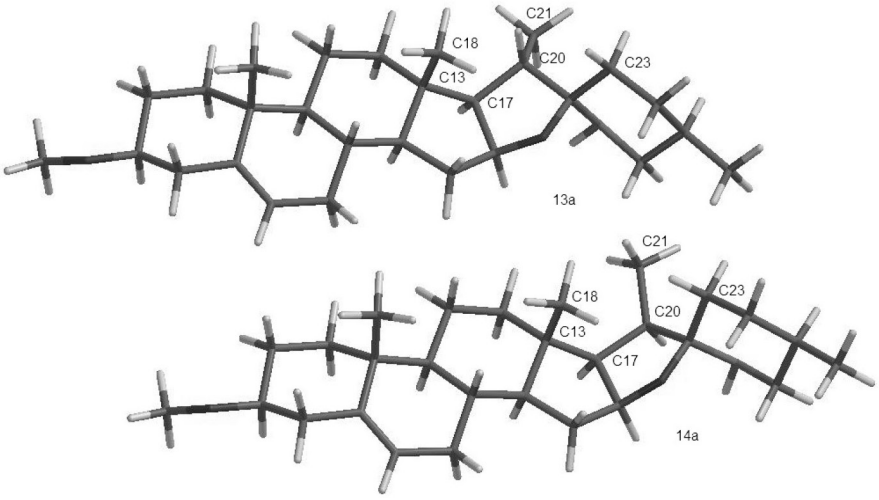

Figure S15. 3D representation of models **13a** and **14a** after optimization with the B3LYP/6-31G* DFT method.