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Synthesis and antifungal activity of medicagenic acid saponins on plant pathogens: modification of the saccharide moiety and the 23α substitution

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ABSTRACT

The study of structure–antifungal activity relationships of medicagenic acid saponins was widened to include synthetic glycosides of mannose, galactose, cellobiose, and lactose as well as a 23α-hydroxymethyl analog of medicagenic acid, namely, methyl 2β,3β-dihydroxy-23α-hydroxymethyl-Δ^{12}-oleanene-28β-carboxylate, against *Sclerotium rolfsii, Rhizoctonia solani, Trichoderma viride, Aspergillus niger,* and *Fusarium oxysporum*. The native glucose-containing saponin was a more effective antifungal agent than the aforementioned saponins, except in the case of the cellobiose-containing derivative and *F. oxysporum*. A carboxyl substituent at the 23α position of the sapogenin brought about higher fungistatic activity than a methyl carboxylate which, in turn, was more effective than an hydroxymethyl group at the same position.

INTRODUCTION

In a study of structure–antimycotic activity relationships of medicagenic acid (**1**) derivatives, it was concluded that *O*-substitution at the 2 or 3 position decreased the activity, while a substitution that may hydrolyse to expose a free 3-OH group (although the extent of this postulated hydrolysis in the experiments conducted was not determined) could be tolerated[1].

While, in some cases it was suggested that the sapogenin rather than the intact saponin is important for the antimycotic activity[2], it was shown in others that the sugar part significantly contributes to the activity[3–5]. Our previous experiments[6] indicated that the glucose-containing saponin of medicagenic acid (G2, **2**) was

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more efficient against dermatophytes than other medicagenic acid derivatives including the maltose derivative of medicagenic acid (F) and medicagenic acid itself. This, however, could not be generalized since, in the case of the plant pathogens, *Fusarium oxysporum* and *Pythium aphanidermatum*, medicagenic acid was clearly more effective than compound G2 although in the former, compound F was even more effective than medicagenic acid[1].

Following an efficient synthesis of the maltose-containing saponin of medicagenic acid that, in general, yields a 3-*O* substitution of this sapogenin[7], it was applied in the present work to additional, novel saponins of the same family and their antimycotic activity was subsequently tested on plant pathogens.

In a recent study, it was demonstrated that enriching the cholesterol or ergosterol content of *Saccharomyces cerevisiae* and of other, medically important, yeasts protected the cells against leakage and killing. On the other hand, an amphotericin B-resistant *Candida tropicalis* which lacks membranal ergosterol, was susceptible[8] to G2. This, alongside with earlier observations that *Pythium*, containing a marginal proportion of sterols[9], was not susceptible to polyene antibiotics (ref 9 and references therein) while it still was susceptible to some extent to saponins (including alfalfa saponins)[1,10]. Furthermore, a conclusion drawn from a comparative study that there appears to be no correlation between the content of sterols and the susceptibility of various plant pathogens to alfalfa saponins[11] suggests that in difference from polyene antibiotics, although complexation of alfalfa saponins with sterols in the cell membranes is an important mechanism for antimycotic activity[12] which may be counteracted by sterols, it is apparently not the only one[8].

EXPERIMENTAL

General methods.—Melting points were determined with a Büchi 510 apparatus. Optical rotations were measured with a Bendix polarimeter. ^{1}H NMR spectra were recorded for solutions in $CDCl_3$, unless otherwise mentioned, on Bruker WH-300 or Varian XL-300 (300 MHz) instruments. IR spectra, for KBr discs, with a Nicolet MX-S FTIR spectrophotometer; UV and colorimetric measurements with a Zeiss PMQ II instrument. Mass spectra were determined on a Jeol SX102 instrument. Photolysis was carried out in a RPR-100 apparatus (Rayonet, The Southern New England Ultraviolet Company, Hamden, CT, USA) in Pyrex glassware. Phenol–H_2SO_4 test (total sugar)[13] was carried out with D-glucose standards.

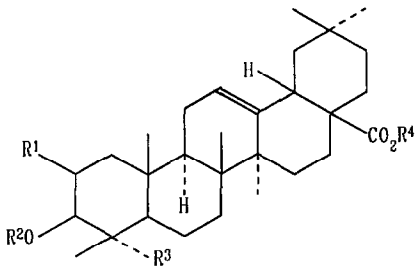
Chromatographic procedures.—TLC analyses were carried out on Silica Gel 60 F_{254} on precoated aluminum plates, purchased from E. Merck, Darmstadt, Germany. Following elution, plates were dried, viewed under UV light or sprayed with 5% (v/v) H_2SO_4 acid in EtOH and heated for 5 min at 110°C to produce color. Column chromatography was carried out using Silica Gel 60 (70–230 mesh) purchased from E. Merck, Darmstadt, Germany. The silica was saturated with the elution solvent prior to application and elution of the column. Preparative HPLC and analytical separations were performed with a Varian LC-5000 liquid chro-

matograph equipped with a preparative Altech RSIL-C-18-HL column (50 × 1 cm) or with a Merck RP-18 (5 μm, 250 × 5 mm) column, respectively. Separations in both cases were monitored by TLC.

Determination and calculation of antifungal activity.—The antifungal activity of medicagenic acid derivatives against plant pathogenic fungi was determined according to the procedure previously described[7,14,15].

Fungal strains.—The fungi tested were supplied by the Department of Phytopathology, Faculty of Agriculture, The Hebrew University of Jerusalem, Israel.

Synthetic saponins of medicagenic acid.—Medicagenic acid (**1**) and compound G2 [**2**, 3β-(β-D-glucopyranosyl)-2β-hydroxy-Δ^{12}-oleanene-23,28-dioic acid], were isolated from alfalfa roots as previously described[14] (modified from ref 16). Di-(2-nitrobenzyl) 2β,3β-dihydroxy-Δ^{12}-oleanene-23,28-dioate was synthesized and condensed by the general procedure of Levy et al.[7] with 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide[17], 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide[18],2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-α-D-glucopyranosyl bromide[19], or 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-glucopyranosyl bromide[20] to yield di-(2-nitrobenzyl) 3-α-*O*-(2,3,4,6-tetra-



1 R^1=OH, R^2=R^4=H, R^3=CO_2H; medicagenic acid
2 R^1=OH, R^2=β-D-Glcp, R^3=CO_2H, R^4=H; compound G2
3 R^1=OH, R^2=2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl, R^3=2-nitrobenzyl carboxylate, R^4=2-nitrobenzyl
4 R^1=OH, R^2=2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl, R^3=2-nitrobenzyl carboxylate, R^4=2-nitrobenzyl
5 R^1=OH, R^2=*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1 → 4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl, R^3=2-nitrobenzyl carboxylate, R^4=2-nitrobenzyl
6 R^1=OH, R^2=*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl, R^3=2-nitrobenzyl carboxylate, R^4=2-nitrobenzyl
7 R=OH, R^2=α-D-Man*p*, R^3=CO_2H, R^4=H
8 R^1=OH, R^2=β-D-Gal*p*, R^3=CO_2H, R^4=H
9 R^1=OH, R^2=*O*-β-D-Glc*p*-(1 → 4)-β-D-Glc*p*, R^3=CO_2H, R^4=H
10 R^1=OH, R^2=*O*-β-D-Gal*p*-(1 → 4)-β-D-Glcp, R^3=CO_2H, R^4=H
11 R^1=OH, R^2=H, R^3=methyl carboxylate, R^4=Me
13 R^1=OH, R^2=H, R^3=hydroxymethyl, R^4=Me
13[a] R^1=acetoxy, R^2=Ac, R^3=acetoxymethyl, R^4=Me
14 R^1=R^2=R^4, R^3=hydroxymethyl; hederagenin

Scheme 1.

TABLE I

Physical properties and analyses of compounds **3–10**

Compound	R_f	t_R [b]	t_R [c]	mp (°C)	$[\alpha]_D^{24}$ (°)	Total sugar (%)	Overall yield [j] (%)
3	0.63 [a]	11.8	71.2	113–116	11.4 [d]		
4	0.63 [a]	11.9	73.0	110–112	42.4 [d]		
5	0.66 [a]	12.4	78.0	82–85	21.3 [d]	26.02 [e]	
6	0.68 [a]	13.1	82.0	88–91	24.6 [d]	25.79 [e]	
7	0.55 [f]			263–265	25.4 [g]	27.16 [h]	26
8	0.38 [f]			233–235	93.3 [g]	27.20 [h]	27
9				173–177	34.6 [g]	43.71 [i]	78
10				154–158	98.6 [g]	43.32 [i]	42

[a] TLC eluted with 3:2 $CHCl_3$–EtOAc. [b] Analytical HPLC employing 82% MeCN in water at 1.5 mL/min. [c] Preparative HPLC employing 80% MeCN in water at 2.0 mL/min. [d] 1% in $CHCl_3$. [e] Calculated value, 25.90%. [f] TLC eluted with 7:2:2 EtOAc–AcOH–H_2O. [g] 1% in water. [h] Calculated value, 27.06%. [i] Calculated value, 43.58%. [j] From the aglycon.

O-acetyl-α-D-mannopyranosyl)-2β-hydroxy-Δ^{12}-oleanene-23,28-dioate (**3**), di-(2-nitrobenzyl) 3-α-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2β-hydroxy-Δ^{12}-oleanene-23,28-dioate (**4**), di-(2-nitrobenzyl) 3β-[*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1 → 4)-*O*-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)]-2β-hydroxy-Δ^{12}-ole-anene-23,28-dioate (**5**), and di-(2-nitrobenzyl) 3β-[*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-*O*-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)]-2β-hydroxy-Δ^{12}-oleanene-23,28-dioate (**6**), respectively. These products were characterized by their physical properties, total sugar analyses (Table I), and particularly by their NMR spectra. ^{1}H NMR: compound **3**: δ 8.08 (m, 2 H, aromatic), 7.66 (m, 4 H, aromatic), 7.41 (m, 2 H, aromatic), 5.65–5.47 (total 4 H, benzylic CH_2), 5.22–3.85 (ms, total 8 H including at δ 5.00, d, 1 H, $J_{1,2}$ 1.0 Hz, H-1), 2.15 (s, 3 H, $OCOCH_3$), 2.11 (s, 3 H, $OCOCH_3$), 2.09 (s, 3 H, $OCOCH_3$), 1.98 (s, 3 H, $OCOCH_3$), and chemical shifts typical to medicagenic acid. Compound **4**: δ 8.07 (m, 2 H, aromatic), 7.62 (m, 4 H, aromatic), 7.50 (m, 2 H, aromatic), 5.63, 5.53, 5.46, and 5.54 (total 4 H, benzylic CH_2), 5.25 (t, 1 H, $J \sim 2.0$ Hz, HC=C), 5.17 (t, 1 H, $J \sim 9.0$ Hz), 5.01 (q, 1 H $J \sim 9.0$, $J \sim 1.5$ Hz), 4.54 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 4.20–3.86 (ms, 4 H), 2.18 (s, 3 H, $OCOCH_3$), 2.07 (s, 3 H, $OCOCH_3$) 2.06 (s, 3 H, $OCOCH_3$), 2.04 (s, 3 H, $OCOCH_3$) and chemical shifts typical to medicagenic acid. Compound **5**: δ 5.36 (t, $J_{4',5'}$ 9.1 Hz, H-4′), 5.25 (t, $J_{3',4'}$ 9.0 Hz, H-3′), 5.08 (t, $J_{3,4}$ 9.0 Hz, H-3), 4.97 (t, $J_{2',3'}$ 9.0 Hz, H-2′), 4.96 (t, $J_{2,3}$ 8.9 Hz, H-2), 4.53 (d, $J_{1,2}$ 8.1 Hz, H-1), 4.42 (d, $J_{1',2'}$ 8.1 Hz, H-1′), 4.17 (dd, $J_{5',6'}$ 3.4 Hz, H-6a′), 4.12 (t, $J_{4,5}$ 9.0 Hz, H-4), 4.06 (dd, $J_{5,6}$ 4.2 Hz, H-6a), 3.98 (dd, $J_{a',b'}$ 12.1 Hz, H-6b′), 3.83 (dd, $J_{a,b}$ 12.1 Hz, H-6b), 3.77 (m, H-5′), and 3.45 (m). (Resonances of protons other than in the carbohydrate moieties are not listed, figures marked and unmarked with primes may be inverted.) Compound **6**: δ 5.37 (dd, $J_{4',5'}$ 0.9 Hz, H-4′), 5.16 (dd, $J_{3',4'}$ 2.4 Hz, H-3′), 5.10 (t, $J_{3,4}$ 9.2 Hz, H-3), 4.99 (dd, $J_{2',3'}$ 10.4 Hz, H-2′), 4.97 (t, $J_{2,3}$ 9.0 Hz, H-2), 4.53 (d, $J_{1',2'}$ 7.9 Hz, H-1′), 4.51 (d, $J_{1,2}$ 8.1 Hz, H-1), 4.19

(dd, $J_{5',6'}$ 6.0 Hz, H-6b'), 4.14 (t, $J_{4,5}$ 9.1 Hz, H-4), 4.07 (dd, $J_{a',b'}$ 10 Hz, H-6a'), 4.04 (dd, $J_{5,6}$ 4.1 Hz, H-6a), 3.92 (m, H-5'), 3.85 (dd, $J_{a,b}$ 12.2 Hz, H-6b), and 3.45 (m, H-5). (Resonances of protons other than in the carbohydrate moieties are not listed, figures marked and unmarked with primes may be inverted.) Compounds **3–6** were irradiated (350 nm) and deacetylated to yield following chromatography[1]: 3β-(α-D-mannopyranosyl)-2β-hydroxy-Δ^{12}-oleanene-23,28-dioic acid (**7**), 3β-(β-D-galactopyranosyl)-2β-hydroxy-Δ^{12}-oleanene-23,28-dioic acid (**8**), 3β-[*O*-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl]-2β-hydroxy-Δ^{12}-oleanene-23,28-dioic acid (**9**), and 3β-[*O*-α-D-galactopyranosyl-(1 → 4)-β-D-glucopyranosyl]-2β-hydroxy-Δ^{12}-oleanene-23,28-dioic acid (**10**), respectively (Table I).

Non-glycosylated derivatives of 2β,3β-Δ^{12}-oleanene.

Methyl 2β,3β-dihydroxy-23α-hydroxymethyl-Δ^{12}-oleanene-28β-carboxylate (**12**).—(*a*) Dimethyl 2β,3β-dihydroxy-Δ^{12}-oleanene-23,28-dioate (**11**, 530 mg, 0.5 mmol)[14] was dissolved in MeOH (20 mL), $NaBH_4$ (37 mg, 1.0 mmol) was added and the solution was kept for 16 h at 4°C. TLC (1:3, $CHCl_3$–EtOAc containing 1% AcOH) examination of the mixture demonstrated the disappearance of the starting material. Ice–water (10 mL) was added to the mixture that was then extracted with cold EtOAc (twice 50 mL). The extract was washed with HCl (50 mL, 1.5 M), $NaHCO_3$ (50 mL, 0.5 M), and water, dried over Na_2SO_4, evaporated and applied to a column of silica (50 g, 2 cm in diameter) and eluted with 1:3, $CHCl_3$–EtOAc containing 1% AcOH. A fraction (R_f 0.33 in TLC, same solvent) was evaporated to yield compound **12** (impure by HPLC, 383 mg, 72% yield); mp 210–212°C; $[\alpha]_D^{25}$ + 34.8° (*c* 1, $CHCl_3$). Comparing the IR spectrum of compound **12** to that of the starting material (**11**), the absorbance at 1724 cm^{-1} (C=O) was reduced to about half.

(*b*) Pure compound **12** was available by deacetylation of compound **13a**. Compound **13a** (50 mg) was dissolved in NaOMe in MeOH (20 mL, 0.1 M), the mixture was kept for 12 h at 4°C, neutralized with HCl (1.5 M), evaporated and purified on a column of silica gel (20 g) as before. The purified product (**12**) had the same mp, IR, and migrated as a single spot in TLC as above.

Methyl 2β,3β-diacetoxy-23α-acetoxymethyl-Δ^{12}-oleanene-28β-carboxylate (**13a**).—Impure compound **12** (200 mg) was treated with pyridine (0.4 mL) and Ac_2O (0.2 mL) for 16 h at 4°C. Ice–water (20 mL) was added and 1 h later, the mixture was extracted with EtOAc (twice 20 mL). The extract was washed with HCl (20 mL, 1.5 M), $NaHCO_3$ (20 mL, 0.5 M), and water, dried over Na_2SO_4 and applied to a column of silica gel (20 g, 2 cm in diameter) eluted with 1:3 $CHCl_3$–EtOAc containing 1% AcOH. Two TLC-pure (same solvent) compounds were isolated: compound **13a**, [R_f 0.90; 102 mg; mp 105°C; $[\alpha]_D^{25}$ + 46.2° (*c* 0.5, $CHCl_3$)] and compound **13b** [R_f 0.79; 3 mg; mp 109–112°C; $[\alpha]_D^{25}$ + 70.6° (*c* 0.5, $CHCl_3$)] that was not further characterized. NMR (pyridine-d_5) and MS data for compound **13a** are as follows: ^{13}C NMR: δ 177.90 (*C*OOMe), 170.42 (CH_3CO), 170.30 (CH_3CO), 170.13 (CH_3CO), 144.31 (C-13), 122.55 (C-12), 72.4 (C-3), 69.69 (C-2), 65.71 (CH_2OH), 51.59, 48.45 (C-9), 48.05, 46.90 (C-19), 45.97 (C-1), 42.05, 41.74, 41.54,

40.48 (C-8), 39.72, 36.87 (C-10), 33.96 (C-15), 33.12, 32.75, 32.62, 30.81, 27.89, 26.08, 23.77 (C-27), 23.65 (C-11), 23.30 (C-30), 21.07, 20.07, 20.62, 18.01, 17.24 (C-25), 16.65 (C-26), and 14.03 (C-24). Assignment of carbons were made[14,21] and found compatible to result in an attached-proton test. ^{1}H NMR includes: δ 5.47 (q, 1 H, J 6.5, J 1.6 Hz), 5.42 (t, 1 H, J 3.2 Hz), 5.24 (apparent t, 1 H, HC=C), 3.74 (s, 3 H, OCH_3), 2.10 (s, 3 H, CH_3CO), 2.09 (s, 3 H, CH_3CO), 2.08 (s, 3 H, CH_3CO), and chemical shifts typical to medicagenic acid. MS (electron impact, 70 eV): m/z 628 (M^+) and 262 (fragment a[22,14]) and 203 (a – $COOCH_3$).

Hederagenin (**14**).—Hederagenin was first characterized in an alfalfa sapogenin preparation[23]. Sapogenin extract from 250 g of alfalfa root flour[14] was applied to a columns of silica gel (200 g, 5 cm in diameter) eluted with 1:3, $CHCl_3$-EtOAc containing 0.5% AcOH (500 mL), followed by 1:4, $CHCl_3$-EtOAc containing 0.5% AcOH (400 mL), and EtOAc (300 mL). The column was monitored by TLC (first solvent), while medicagenic acid (**1**, 2 g) was isolated from fractions with R_f 0.25, hederagenin (**14**, 85 mg) emerged in fractions with R_f 0.30.

RESULTS AND DISCUSSION

Medicagenic acid and its naturally-occurring glycosides present in alfalfa are affective antifungal agents that may be used in plant protection and in therapy.

Four medicagenic acid (**1**) saponins (compounds **7–9**), synthesized in this work[14] represent different saccharide 3-*O*-substitution of the aglycon. This includes compounds **8** and **9** that were synthesized in this work as well as before and were found active against *S. rolfsii*, although only minimal experimental details were available[24]. In addition, compound **12**, where the 23α-methyl carboxylate grouping was reduced by sodium borohydride in polyethylene glycol[25] or in similar yields by sodium borohydride in methanol (see Experimental) was made.

Important support for the structures assigned to compounds **3–10**, **12**, and **12a** is available from the ^{1}H NMR particularly concerning the anomeric nature of the newly-formed glycosidic bonds. Thus, $J_{1,2}$ 1.0 Hz for compound **3** is compatible with an *e,e* interaction and fits an α-D-mannopyranoside structure while $J_{1,2}$ 7.9, 8.1, or 7.9 Hz in the cases of compounds **4–6**, respectively, is compatible with *a,a* interactions that are expected in compounds possessing only β-D-glucopyranosyl and β-D-galactopyranosyl residues. The ^{1}H and ^{13}C NMR spectra of compound **13a** show that one methyl carboxylate function present in compound **11** alongside with two hydroxy functions were converted in into acetoxy functions. This does not indicate, however, which methyl carboxylate (of the two present in compound **11**) was reduced. The information required for discriminating between the two possibilities follows from the fragmentation in the mass spectrum; m/z 262 fits an acetoxymethyl substitution on C-23 while a similar substitution on C-28 of the aglycon should have given rise to m/z 276.

Already, comparing the maltoside (compound F) to the glucoside (**2**) of medicagenic acid (**1**), it was concluded that the presence of the sugar component at the 3β

Table II

Maximal percent inhibition of plant pathogenic fungi by some derivatives of medicagenic acid [a]

Fungus	1		2		7	8	9	10	11		12	14
S. rolfsii	80(66) [b]	82(75) [c]	68(67) [b]	84(75) [c]	57(67) [b]	52(22) [b]	32(75) [c]	63(75) [c]	78(66) [b]	86(75) [c]	43(66) [b]	58(92) [b]
R. solani	96(66) [b]	83(31) [c]			87(22) [b]	54(22) [b]			53(27) [b]	53(35) [c]	49(19) [b]	58(27) [b]
T. viride	99(43) [b]	93(75) [c]	91(45) [b]	87(75) [c]	90(56) [b]	75(22) [b]	68(75) [c]	65(75) [c]	49(38) [b]	62(75) [c]	42(19) [b]	52(53) [b]
A. niger	62(43) [b]	51(150) [c]		52(150) [c]			62(150) [c]	63(150) [c]	40(19) [b]	37(150) [c]	37(38) [b]	47(28) [b]
F. oxysporum	87(79) [b]	81(150) [c]		51(150) [c]			78(150) [c]	77(150) [c]	75(66) [b]	67(66) [c]	67(79) [b]	69(66) [b]
P. aphanidermatum	58(52) [b]	61(31) [c]							100(27) [b]		86(27) [b]	72(27) [b]

[a] Joham[27] medium, incubation temperature 26 ± 1°C, percent inhibition produced by compound concentration of 40 μg/mL, numbers in parentheses represent incubation time (h); for experimental details, see Levy et al.[14] and Zehavi et al.[15]. [b] Set of experiments performed simultaneously. [c] Set of experiments performed simultaneously.

position affected the antimycotic activity[7]. It was, therefore, of interest to compare the antimycotic activity of compounds **2** and **7–10** that have a common aglycon, medicagenic acid (**1**), but differ in their saccharide moieties. The inhibition of plant pathogenic fungi by these derivatives (Table II) suggests that compounds **7–10** had no advantage over compound **2** with the exception of compound **9** and *F. oxysporum*. Assuming that two factors might influence the antifungal activity of compounds **2** and **7–10**, namely, the altered hydrophilicity affecting transport to the site of action and susceptability to glycosidases of the fungus[7], no further evaluation of the results is possible without a determination of the relative hydrophilicity of the aforementioned compounds and of the different glycosidases activities. Generally, if calculated on a molar basis, the saponins were more active than the sapogenin (**1**) and only in one case (compound **9** and *S. rolfsii*), the presence of a cellobiosyl substitution significantly reduced the activity.

Compound **12** has been synthesized to evaluate the importance of the 23α-carboxyl or methyl carboxylate in medicagenic acid. The relative activity in cases other than *P. aphanidermatum* has been **1** > **11** > **12**, indicating that the antimycotic effectiveness of substitution of the 3β position appears to be COOH > $COOCH_3$ > CH_2OH (compound **12** had a higher maximal percent inhibition of 100% at 20 μg/mL — equal to that of compound **13**, data not shown). The information presented (Table II) further supports the notion that the presence of a carboxylic acid (or an ester that may hydrolyze to the free acid) at the 23α position is important to the activity, in addition to the structure–antimycotic activity relationships suggested in the past[1]. In the present work, it was observed that the time required to achieve maximal inhibition at lower inhibitor concentration (10 μg/mL, data not presented) was shorter for medicagenic acid saponins compared to other derivatives of medicagenic acid. In general, in this series of compounds, differences in the antifungal activity may be interpreted *inter alia* by differences in penetration into the fungal cells, extent of interaction with membranal sterols and cell components, hydrolytic and detoxification activities of the fungi and the host tissues.

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