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A LABDANE DITERPENE AND ITS GLYCOSIDE FROM *MELODINUS MONOGYNUS*

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Key Word Index—*Melodinus monogynus*; Apocyanaceae; medigenin; medinin; diterpene; labdane.

Abstract—A novel labdane diterpene, medigenin, and its glycoside, medinin, have been isolated from the dried root bark of *Melodinus monogynus*. On the basis of chemical and spectroscopic data the structures have been established as 16,19-dihydroxy-entlabda-8(17)13-diene-15-oic lactone and its 19-*O*-β-cellobioside, respectively.

INTRODUCTION

Chemical screening of the root bark of *Melodinus monogynus* showed it to be rich in butenolides. An aqueous concentrate of the alcoholic extract of the root bark when shaken with ether gave an ether-soluble residue containing a mixture of genins and glycosides. The isolation of medigenin and its glycoside medinin from the ether extract was reported earlier by Khare *et al.* [1], who showed them to be α,β-unsaturated butenolide derivatives and established that the sugar moiety of medinin was D-glucose. It was, therefore of interest to undertake the structural elucidation of these compounds.

RESULTS AND DISCUSSION

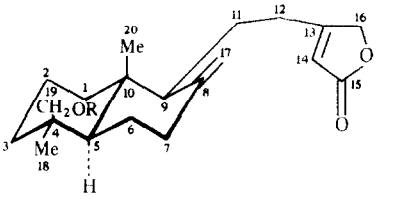
Medigenin (**1**), molecular formula $C_{20}H_{30}O_3$ (elemental analysis and $[M]^+$), gave a pink colouration in the Kedde [2, 3] reaction indicative of the presence of a butenolide ring in the molecule. This was also supported by characteristic absorption bands in its IR (1795 and 1730 cm^{-1}) and UV λ_{max} (ethanol) 214 nm (log ε 4.14) spectra. A prominent band in its IR spectrum at 3485 cm^{-1} indicated the presence of a hydroxyl group, while two other prominent bands at 1645 and 900 cm^{-1}, attributable to C=C stretching and vinyl C–H bending vibrations, suggested the presence of an exocyclic methylene group. The presence of an exocyclic methylene group containing an isolated double bond was in agreement with the positive colour reaction with tetranitromethane reagent [4, 5].

As acetylation of medigenin with acetic anhydride in pyridine gave the monoacetate **5**, $C_{22}H_{32}O_4$, this suggested the presence of only one acetylateable hydroxyl group in the parent molecule.

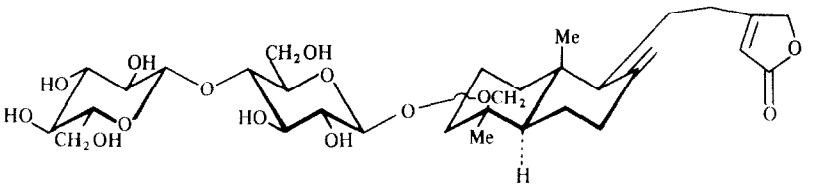
The DBE value of **1** was calculated as six. After assigning a DBE value of three to the butenolide ring and a value of one to the exocyclic methylene double bond, medigenin had to contain two rings. The molecular formula of medigenin ($C_{20}H_{30}O_3$) and its bicyclic nature suggested it was a monohydroxy diterpene butenolide possessing a decalin skeleton presumably belonging to the labdane group. The isolation of this class of compounds from other plants, (i.e. compound $C_{20}H_{30}O_4$ from *Xanthoceptialum linearifolium* [6], andrographolide [7], $C_{20}H_{30}O_5$, and its glycoside neoandrographolide [8] from *Andrographis paniculata*) had been reported earlier. Although neoandrographolide was assigned a skeletal structure similar to the glucopyranoside of medigenin, on acid hydrolysis it did not afford the expected medigenin as the isolated isoaglucone had its exocyclic methylene group isomerised to a C-8, C-9 double bond under the hydrolytic conditions employed as a result of which the isolated product possessed mp and rotation different from medigenin.

A careful analysis of the ^1H NMR spectrum of **1** supported the presence of a labdane diterpene moiety in medigenin. Two, three-proton singlets appearing at δ0.68 and 1.0 were assigned to C-10 and C-4 tertiary methyl groups with the unusual higher field singlet at δ0.68 being attributed to the highly shielded axial C-10 methyl group of the labda-8-en skeleton [9, 10]. Two broad singlets of one proton each at δ 4.49 and 4.90 were in close accord with the reported values for each of the exocyclic methylene group protons. Two doublets of one proton each at δ 3.40 ($J = 11$ Hz) and 3.82 ($J = 11$ Hz) were characteristic of the methylene protons of an axial-CH_2OH group at C-4 [11], suggesting a methyl and hydroxymethyl group at C-4. A one proton triplet centered at δ5.86 ($J = 2$ Hz, H-14) indicative of α-olefinic proton along with a two proton doublet at δ4.73 ($J = 2$ Hz, H-16) suggested the presence of an α,β-unsaturated γ lactone group [12] in agreement with the UV data.

In the EI mass spectrum of **1**, $[M]^+$ appeared at m/z 318 ($C_{20}H_{30}O_3$). That one of the three oxygen atoms of **1** was present as a hydroxyl group and the remaining two oxygen were involved in the butenolide group was confirmed by the presence of peaks at *m/z* 300 (**6**) $[M-H_2O]^+$ and 189 (**7**). The mass spectral fragmentation pattern of **1** was consistent with the presence of labda-8-en skeleton in the molecule as shown by the diagnostic fragment ion peak at *m/z* 220 attributable to the formation of the bicyclic nucleus **8** from the labdane moiety [11, 14] and further substantiated by a peak at *m/z* 98 due to the butenolide moiety. Fragment ions at *m/z* 287 (**9**) and 272 corresponded to the successive losses of the tertiary hydroxy methyl group $[M-CH_2OH]^+$ and methyl group $[M-CH_2OH-Me]^+$, respectively. Moreover, in



1 R = H
5 R = Ac



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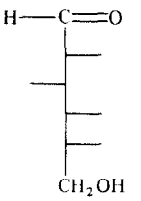

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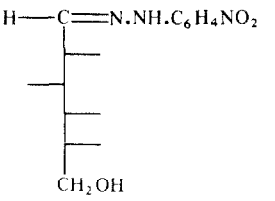

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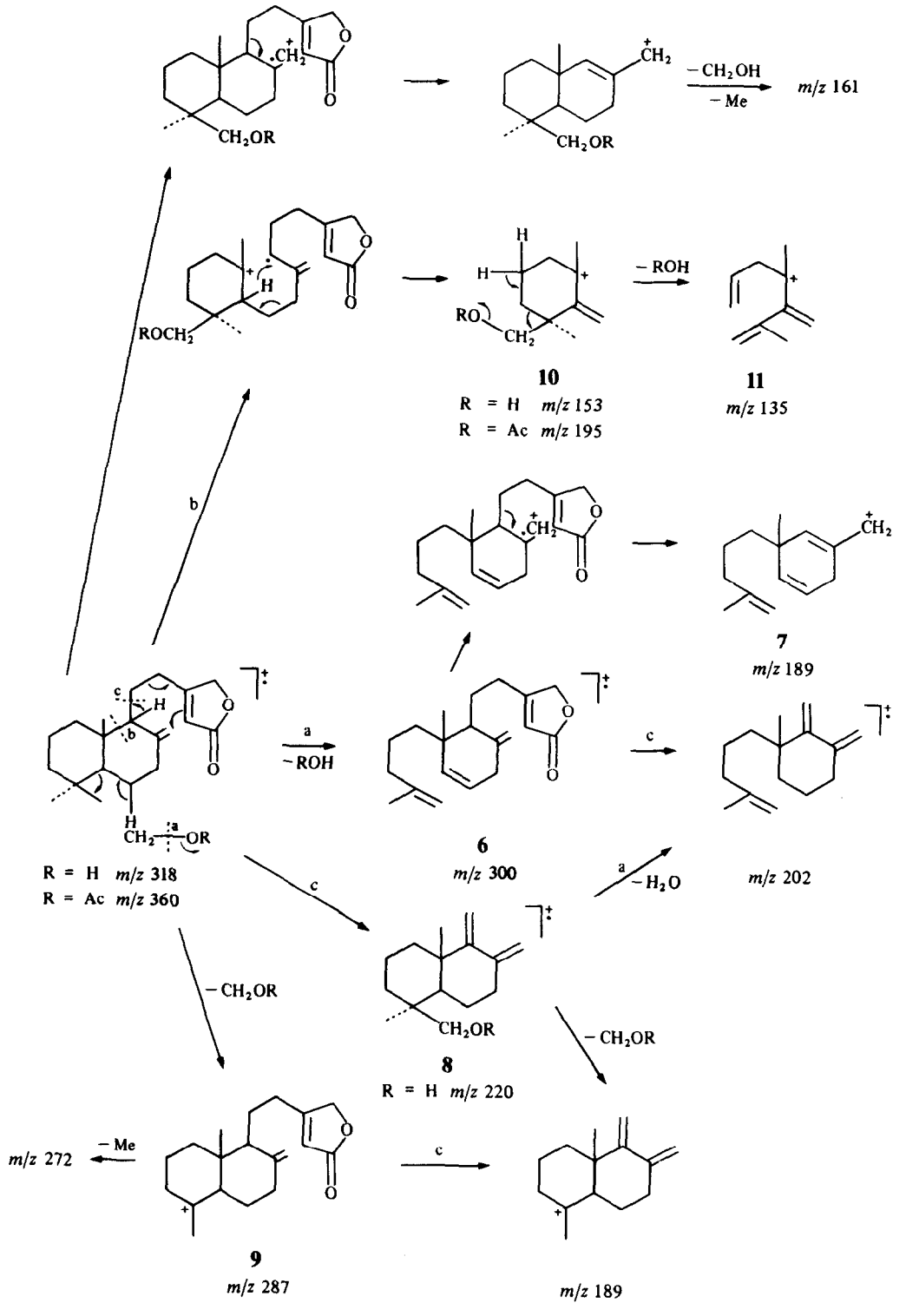
a bicyclic diterpene containing an exocyclic methylene group allylic cleavage [15, 16] is known to give a prominent peak at *m/z* 153 **(10)** as observed in the mass spectrum of **1**. This ion originates from ring A by fission of bond C-6, C-7 and C-9, C-10 and supports the presence of two methyl and one hydroxymethyl group in ring A. The subsequent loss of a water molecule to give a peak at *m/z* 135 **(11)** $[153-H_2O]^+$ indicated the axial nature of CH_2OH group [17].

Acetylation of **1** with acetic anhydride in pyridine afforded a monoacetate **5** which gave no hydroxyl absorption band in its IR spectrum suggesting the presence of only one acetylateable hydroxyl group in **1**. This was further supported by a three proton acetoxy group singlet at δ2.10 in its ^{1}H NMR spectrum. Besides this, the two doublets of one proton observed in **1** were each shifted to lowerfield by *ca* 0.5 ppm to δ3.89 (J = 10.5 Hz) and 4.27 (J = 10.5 Hz). This was in agreement with the axial configuration of the acetoxymethyl group at C-4 in the molecule [7].

The mass ion peak at *m/z* 360 in the mass spectrum of **5** was in agreement with the formula $C_{22}H_{32}O_4$, indicating it to be a monoacetate. The mass ion peak at *m/z* 300 **(6)** $[M-AcOH]^+$ was attributed to the loss of one molecule of AcOH. Other ions characteristic of allylic cleavage of C-6, C-7 and C-9, C-10 bonds of the labda-8-en moiety gave peaks at *m/z* 195 and 135 **(11)** $[195-AcOH]^+$ (base peak). The peak recorded at *m/z* 189 could be accounted for by the loss of CH_2OAc group. On the basis of above chemical and spectroscopic data, it was concluded that **1** is 16,19-dihydroxy-entlabda-8(17)13-diene-15-oic lactone.

Medinin **(2)**, $C_{32}H_{50}O_{13}$, exhibited a positive colour reaction for a butenolide ring in the Kedde reaction [2, 3]. It gave the colour response of a normal sugar in the Fiegl test [18] and this in conjunction with its non-reducing property (Fehlings test) suggested it to be a normal sugar glycoside. Absorption bands at 1750 and 1632 cm^{-1} in the IR spectrum of **2** characteristic of a butenolide ring and at λ_{max} 213 nm ($\log \varepsilon = 4.09$) in its UV spectrum suggested it to be normal sugar glycoside of a butenolide. To characterize its sugar and genin components, it was acid hydrolysed by the method of Mannich and Siewert [19, 20] yielding medigenin (mmp, rotation and co-chromatography on PC with the earlier isolated sample of **1**). The sugar component crystallized from alcohol–water afforded **3** which was characterized as D-glucose [21, 22] (mp, mmp, rotation and co-chromatography on PC). For further characterization, the sugar was converted into a crystalline *p*-nitrophenyl hydrazone **4** [23] which had the same properties as an authentic specimen of D-glucose *p*-nitrophenyl hydrazone. The difference of $C_{12}H_{20}O_{10}$ between the formula of medinin **(2)** ($C_{32}H_{50}O_{13}$) and medigenin **(1)** ($C_{20}H_{30}O_3$) suggested that **2** was diglycoside. The disaccharide moiety could be either cellobiose or maltose. The molecular rotation of medinin (found: -166°, calcd: -232°) [calculated from that of (-143.1°) of medigenin and (-89°) of methyl β-D-cellobioside] was found to agree with the Klyne rule [24] and suggested medinin was a glycoside of β-cellobioside and not α-cellobioside for which the calculated value of the glycoside comes to $+201^\circ$ (Table 1).

On the basis of the preceding data it was concluded that medinin **(2)** is the β-cellobioside of **1**.



Biological activity

Medigenin (**1**), medigenin acetate (**5**) and medinin (**2**) showed cardiotonic activity with (a) isolated frog heart perfused with Ringer solution and (b) isolated mammalian (rabbit) heart perfused with Ringer–Lockes solution isolated by Anderson-Coronary perfusion apparatus.

On the isolated frog heart, medigenin (**1**) increased the tone and force of contraction of the heart and the heart rate was decreased. Similarly a positive inotropic and negative chronotropic effect was observed on the isolated rabbit heart. The acetylated derivative (**5**) also increased the tone and force of myocardial contraction and de-

Table 1

Disaccharide	Genin ϕ	Calcd diglycoside ϕ	Found medinin ϕ
Cellobiose			
α-Methyl + 344.6	−143.1	+201.5	−166.42
β-Methyl − 89	−143.1	−232.1	
Maltose			
β-Methyl + 651.4	−143.1	+508.3	−166.42
α-Methyl +270.5	−143.1	+127.4	

creased the heart rate. Medinin **(2)** showed no cardiac activity with either of the heart preparations.

These results indicated that medigenin and medigenin acetate have definite cardiotonic activity whereas the diglucoside medinin is devoid of activity. Medigenin acetate is more potent than medigenin.

EXPERIMENTAL

Mps: uncorr. Sugars were visualized with 50% aq H_2SO_4 (TLC) or partridge reagent (PC). PC was performed using. $CHCl_3$–$HCONH_2$.

Plant extraction. Shade dried roots (10 kg) of *M. monogynus* were extracted and fractionated as reported earlier [1]. Repeated CC of the Et_2O extract (2.08 g) over silica using C_6H_6–$CHCl_3$ as eluent afforded medigenin (65 mg) and medinin (45 mg).

Medigenin **(1).** Mp 165–167° (Me_2CO–nC_5H_{12}), $[\alpha]_D^{25}$ −45° (MeOH; *c* 1.0). Elemental analysis: $C_{20}H_{30}O_3$ [1]. Pink colour with Kedde reagent. UV λ_{max}^{EtOH} nm (log ε): 214 (4.14); IR ν_{max}^{KBr} cm^{-1} 3485 (OH), 1645 and 900 (exocyclic methylene), 1795 and 1730 (α,β-unsaturated lactone); ^1H NMR: δ5.86 (1H, *t*, *J* = 2 Hz, H-14), 4.73 (2H, *d*, *J* = 2 Hz, H-16), 4.9 (1H, *br s*, $-C{=}CH_2$, H-17) 4.49 (1H, *br s*, $-C{=}CH_2$, H-17), 3.82 (1H, *d*, J = 11 Hz, H-19), 3.40 (1H, *d*, *J* = 11 Hz, H-19), 1.0 (3H, *s*, Me-18), 0.68 (3H, *s*, Me-20); MS: *m/z* (rel. int.): 318 $[M]^+$ (23), 300 (18) $[M-H_2O]^+$, 287 (45) $[M-CH_2OH]^+$, 272 (30) $[M-CH_2OH-Me_3]^+$, 220 (47) [fragment **8**, $C_{15}H_{24}O]^+$, 202 (57) $[\mathbf{8}-H_2O]^+$, 189 (21) $[\mathbf{8}-CH_2OH]^+$, 161 (28) $[M-111-CH_2OH-Me_3]$ 153 (30) [fragment **10**, $C_{10}H_{17}O]^+$, 135 (70) $[\mathbf{10}-H_2O]^+$, 123 (100) $[C_9H_{15}]^+$.

Mono-O-acetyl medigenin **(5).** A soln of **1** (10 mg) in pyridine (0.3 ml) and Ac_2O (0.3 ml) was kept for 24 hr at room temp. Usual work-up yielded mono-*O*-acetyl medigenin **(5)** which crystallized as colourless plates (10 mg) from cyclohexane. MP 96–98°, $[\alpha]_D^{25}$ −46° (MeOH, *c* 1.0). Elemental analysis: $C_{22}H_{32}O_4$. Pink colour in Kedde reaction. UV λ_{max}^{EtOH} nm (log ε): 214 (4.14); IR ν_{max}^{KBr} cm^{-1} 1645 and 891 (exocyclic methylene) 1783, 1740 (α,β-unsaturated-γ-lactone); ^1H NMR: δ5.85 (1H, *t*, *J* = 2 Hz, H-14), 4.73 (2H, *d*, *J* = 2 Hz, H-16), 4.92 (1H, *br s*, $-C{=}CH_2$, H-17), 4.52 (1H, *br s*, $-C{=}CH_2$, H-17), 4.27 (1H, *d*, *J* = 10.5 Hz, H-19), 3.89 (1H, *d*, *J* = 10.5 Hz, H-19), 2.10 (3H, *s*, OAc), 1.0 (3H, *s*, Me-18), 0.75 (3H, *s*, Me-20); MS: *m/z* (rel. int.): 360 $[M]^+$ (12), 300 (75) $[M-HOAc]^+$, 287 (74) $[M-CH_2OAc]^+$, 272 (25) $[M-CH_2OAc-Me]^+$, 195 (4) [fragment **(10)**, $C_{12}H_{19}O_2]^+$, 189 (30) [fragment $\mathbf{8}-CH_2OAc$], 161 (37) $[M-111-CH_2OAc-Me]^+$, 135 (100) $[195-HOAc]^+$.

Medinin **(2).** Mp 120–122°, ($CHCl_3$–Me_2CO/H_2O), $[\alpha]_D^{25}$ −26° (MeOH, *c* 1.0). Elemental analysis: $C_{32}H_{50}O_{13}$. Pink colour in Kedde as well as Feigl test. UV λ_{max}^{EtOH} nm (log ε), 213 (4.09); IR ν_{max}^{KBr} cm^{-1} 3380 (OH groups), 1750, 1632 (α,β-unsaturated-γ-lactone).

Mannich hydrolysis of **2** *with acid.* To a soln of crystalline **2** (25 mg) in Me_2CO (2.5 ml), conc HCl (0.075 ml) was added. The soln was kept under CO_2 in a dark room at room temp. for 75 hr. To the hydrolysate, 3 ml H_2O was added and the Me_2CO removed under red. pressure. To the aq. soln, MeOH (3 ml) was added and refluxed on a water bath for 20 min and the MeOH removed by distillation. The aq. portion was repeatedly extracted with $CHCl_3$ and the organic layer was washed in turn with H_2O, Na_2CO_3 and H_2O, dried over Na_2SO_4 and evapd to afford **1** (10 mg), mp 164–166° (Me_2CO–nC_5H_{12}), $[\alpha]_D^{25}$ −44° (MeOH, *c* 1.0), pink colour with Kedde reagent. The aq. hydrolysate was neutralized with freshly prepared Ag_2CO_3 and filtered. The filtrate was cooled in ice and H_2S passed to remove Ag^+ ions as Ag_2S and filtered. The neutral aq. filtrate was concd under red. pressure to afford the syrupy sugar **3** (8 mg) which crystallized from EtOH–H_2O. MP 80–82°, $[\alpha]_D^{25}$ +99° (H_2O, *c* 1.0), pink colouration in Feigl reaction. Comparison of its optical rotation and co-chromatography (PC) identified **3** as D-glucose.

p-*Nitrophenyl hydrazone of sugar* **3.** A mixture of 5 mg *p*-nitrophenyl hydrazine and crystalline sugar (5 mg) was dissolved in EtOH (2 ml) and 2 drops HOAc added. The mixture was heated on a water bath for 2 hr. The viscous mass was cooled in ice and the pptd *p*-nitrophenyl hydrazone **4** recovered by filtration and crystallized from EtOH–H_2O. MP 192°.

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