present at C-23 rather than at C-21 as the olefinic proton at δ7 2 could be assigned to that at C-22 (β to the carbonyl) The chemical shifts [7] of the isomeric hydroxybutenolide (4) having a hydroxyl group at C-21 and a carbonyl group at C-23 synthesized during the preparation of cardenolides from furyl androstanes [7] are reported to be δ5 8 and 5 9 for the C-21 and C-22 protons, respectively This fully established the structure of salannolide as 1

This is the first report of the natural occurrence of a hydroxybutenolide side chain present in a meliacin and it is of biogenetic significance This compound could be related to the dihemiacetal (5), prepared [8] by degradation of turreanthin, as an intermediate in the possible route for the formation of the furan ring in meliacins

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ISOLATION AND STRUCTURES OF DIOMUSCINONE AND DIOMUSCIPULONE FROM *DIONAEA MUSCIPULA*

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Key Word Index—*Dionaea muscipula*, Droseraceae, phenolic compounds, diomuscinone, diomuscipulone

Abstract—From the fresh leaves and roots of *Dionaea muscipula*, two new substances (diomuscinone and diomuscipulone) have been isolated together with the known naphthoquinone plumbagin The structures of the new compounds have been elucidated on the basis of their spectral data coupled with some chemical evidence

INTRODUCTION

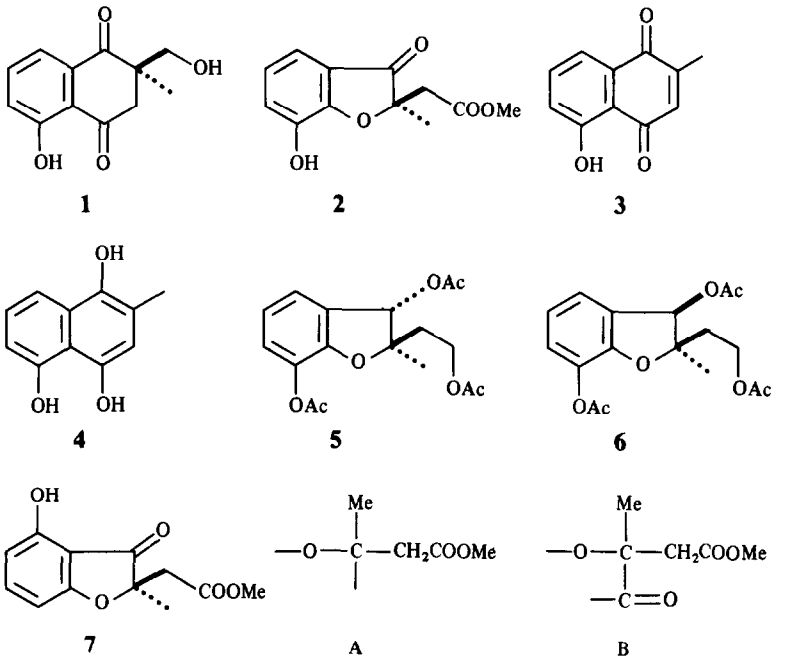
From *Dionaea muscipula* E, we have isolated two new interesting compounds named diomuscinone (1) and diomuscipulone (2), in addition to the known naphthoquinone, plumbagin (3) [1, 2] From a biogenetic point of view, the newly isolated substances (1 and 2) seem to be related to plumbagin (3), which is the principal component This paper describes the isolation and structures of diomuscinone and diomuscipulone Furthermore, the biogenetic relationship between diomuscipulone (2) and plumbagin (3) is also demonstrated

RESULTS AND DISCUSSION

The ethyl acetate soluble part of the methanol extract of *D muscipula* was separated by a combination of silica gel column chromatography and preparative TLC to afford diomuscinone (1), diomuscipulone (2) and plumbagin (3) [1, 2], in 0 024, 0 014 and 2 1% yields (from weight of the methanol extract), respectively

Diomuscinone (1), molecular formula $C_{12}H_{12}O_4$, has two CO groups (δ200 8 and 202 8) and a tri-substituted aromatic ring (δ118 7, 124 0 and 136 9) The presence of an Me–C(–)–CH_2OH grouping is suggested on the basis of its ^1H and ^{13}C NMR spectra [δ1 31 (3H, *s*), 3 53 (1H, *d*, J = 11 5 Hz) and 4 06 (1H, *d*, J = 11 5 Hz), δ21 4 (*q*), 50 5 (*s*) and 67 8 (*t*)] On the basis of these and other spectral data and the following chemical evidence together with co-occurrence of plumbagin (3) as a main component, the structure of diomuscinone must be represented by 1 When treated with 60% sodium hydride in mineral oil at room temperature for 12 hr, diomuscinone (1) is readily converted into plumbagin (3), in 82% yield, via the corresponding hydroquinone-type intermediate (4).

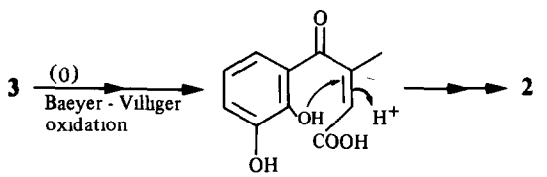
Diomuscipulone (2), with a molecular formula $C_{12}H_{12}O_5$, has one ketonic carbonyl group (1700 cm^{-1}), one hydroxyl group and a tri-substituted aromatic ring [δ6 85–7 25 (3H, complex)] Its ^1H NMR and mass spectra {δ1 43 (3H, *s*), 2 99 (2H, *s*) and 3 50 (3H, *s*), *m/z* 177 $[M-COOMe]^+$ and 163 $[M-CH_2COOMe]^+$} show that it contains partial structure A, which must be further extended to B on the basis of the following chemical



evidence On reduction with lithium aluminium hydride in THF (room temp, 15 hr) followed by acetylation with acetic anhydride–pyridine (room temp, 15 hr), diomuscipulone (**2**) is readily converted into two triacetates (**5** and **6**) in 38 and 17% yields, respectively The stereochemistry of the newly formed secondary acetate group in **5** and **6** is unambiguously established from their ^{1}H NMR spectra the methylene signal in **5** [δ4 20 (2H, *t* (*br*), J = 7 Hz)] is observed upfield from that in **6** [δ4 29 (2H, *t*, J = 7 Hz)] whereas the methyl singlet in **5** (δ1 46) appears downfield from the corresponding one in **6** (δ1 40) because of the deshielding effect of the secondary acetate group, indicating that the methyl group in **5** and the methylene group in **6** are both *cis* to the secondary acetate group From these results, two possible structures (**2** and **7**) are assignable to diomuscipulone However, the former (**2**) is the more favourable on the basis of the IR absorption band at 1700 cm^{-1} due to the carbonyl group, indicating that there is no intramolecular hydrogen bonding This is also in good agreement with the biogenetic relationship between diomuscipulone (**2**) and plumbagin (**3**), shown in Scheme 1

EXPERIMENTAL

Mps are uncorr ^{1}H NMR (90 MHz) and ^{13}C NMR (50 MHz) spectra were taken in $CDCl_3$ or in Me_2CO-d_6 with TMS as internal standard Mass spectra (70 eV) were measured with a direct inlet system



Scheme 1 Biogenetic relationship between **2** and **3**

Extraction and isolation The fresh leaves and roots of *D muscipula* (4 5 kg), which were collected late in April, were pulverized with a mixer and immersed in MeOH (10 l × 2) for 6 days, and then filtered The filtrate was concd under red pres to leave a greenish residue (192 g) which was partitioned between H_2O (800 ml) and EtOAc (800 ml × 3) The combined EtOAc soln was concd under red pres to give an oil (48 g), which was subjected to CC on silica gel (Wakogel C-200) (750 g) using hexane–EtOAc (4 1) From the first two fractions (*ca* 1000 ml), plumbagin (**3**, 4 0 g) as orange needles was obtained in pure state (mp, IR and ^{1}H NMR spectra) [1] Further elution with the same solvent system (*ca* 1000 ml) afforded a syrup (1 65 g) which was further separated by repeated prep TLC (Kieselgel 60 F_{254}, 0 5 or 2 mm) using C_6H_6–EtOAc (8 1) and then hexane–EtOAc (2 1) to afford diomuscipulone (**2**, 26 mg), mp 178–179° (from hexane–Me_2CO) IR ν_{max}^{film} cm^{-1} 3200 (*br*), 1735, 1700 (*br*), 1625, 1595, ^{1}H NMR (Me_2CO-d_6) δ1 43 (3H, *s*), 2 99 (2H, *s*), 3 50 (3H, *s*), 6 85–7 25 (3H, complex), MS *m/z* (rel int) 236 $[M]^+$ (100), 204 (35), 177 (66), 163 (40), 137 (32), MS *m/z* 236 0677 $[M]^+$, calc for $C_{12}H_{12}O_5$ *m/z* 236 0683

Further elution with hexane–EtOAc (2 1, 500 ml) and then with hexane–EtOAc (1 1, *ca* 1000 ml) afforded an oil (390 mg) which was purified by repeated prep TLC (Kieselgel 60 F_{254}, 0 5 or 2 mm) using C_6H_6–EtOAc (5 1), $CHCl_3$–MeOH (9 1) and then C_6H_6–EtOAc (4 1) to afford diomuscinone (**1**, 46 mg), mp 85–85 5° (from hexane–C_6H_6) IR ν_{max}^{film} cm^{-1} 3430 (*br*), 1670, 1640, 1600, 1575, 1510 (*br*), 1490, ^{1}H NMR ($CDCl_3$) δ1 31 (3H, *s*), 2 80 (1H, *d*, J = 17 Hz), 3 43 (1H, *d*, J = 17 Hz), 3 53 (1H, *d*, J = 11 5 Hz), 4 06 (1H, *d*, J = 11 5 Hz), 7 25–7 4 (1H, overlapped with the solvent signal), 7 5–7 8 (2H, complex), ^{13}C NMR ($CDCl_3$) δ21 4 (*q*), 46 6 (*t*), 50 5 (*s*), 67 8 (*t*), 117 5 (*s*), 118 7 (*d*), 124 0 (*d*), 134 0 (*s*), 136 9 (*d*), 161 1 (*s*), 200 8 (*s*), 202 8 (*s*), MS *m/z* (rel int) 220 $[M]^+$ (97), 202 (9), 191 (100), 190 (82), 189 (68), 175 (34), 163 (38), 120 (34), 92 (30), MS *m/z* 220 0704 $[M]^+$ (calc for $C_{12}H_{12}O_4$ 220 0734)

Conversion of diomuscinone (**1**) *to plumbagin* (**3**) To a soln of **1** (5 mg) in THF (2 ml) was added 60% NaH in mineral oil (5 mg) at

0°, with stirring, under Ar The soln was stirred at room temp. for 15 hr, and then made acidic with excess AcOH and concd under red pres to leave a syrup, which was partitioned between EtOAc and H_2O The EtOAc soln was washed with aq satd NaCl and then dried (Na_2SO_4) After filtration, the filtrate was concd under red pres and then purified by prep TLC (Kieselgel 60 F_{254}, 0 5 mm) using hexane–EtOAc (1 1) to give orange needles of plumbagin (3, 3 5 mg) (mp, TLC and IR spectrum)

Conversion of diomuscipulone (2) *to triacetates* 5 *and* 6 To a soln of 2 (5 mg) in THF (2 ml) was added $LiAlH_4$ (10 mg), with stirring, at 0°, and then the reaction soln was further stirred at room temp for 15 hr After acidification with 2 N HCl, the reaction mixture was diluted with H_2O and extracted with EtOAc The EtOAc soln was washed with aq satd NaCl, and then dried (Na_2SO_4) After filtration, the filtrate was concd under red pres to give an oil, which was dissolved in Ac_2O–pyridine (1 1, 1 ml) and allowed to stand at room temp for 15 hr The reaction soln was concd under red pres to give an oily residue, which was separated by repeated prep TLC (Kieselgel 60 F_{254}, 0 5 mm) using hexane–EtOAc (1 1) and then $CHCl_3$–EtOAc (9 1) to afford 5 (2 7 mg) and 6 (1 2 mg) Compound 5, colourless oil IR ν_{max}^{film} cm^{-1} 1775, 1740, 1620, 1600, 1485, ^1H NMR ($CDCl_3$) δ1 46 (3H, *s*), 2 00 (3H, *s*), 2 07 (3H, *s*), 2 1–1 95 (2H, overlapped with OAc signals), 2 30 (3H, *s*), 4 20 (2H, *t* (*br*), J = 7 Hz), 6 10 (1H, *s*), 6 9–7 3 (3H, complex, overlapped with the solvent signal), MS *m/z* (rel int) 336 $[M]^+$ (90), 294 (100), 277 (36), 252 (33), 250 (93), 234 (74), 217 (45), 208 (29), MS *m/z* 336 1204 $[M]^+$, calc for $C_{17}H_{20}O_7$ *m/z* 336 1208 Compound 6, colourless oil IR ν_{max}^{film} cm^{-1} 1775, 1740, 1610, 1600, 1485, ^1H NMR ($CDCl_3$) δ1 40 (3H, *s*), 2 03 (3H, *s*), 2 07 (3H, *s*), 2 28 (3H, *s*), 2 0–2 3 (2H, overlapped with OAc signals), 4 29 (2H, *t*, J = 7 Hz), 5 97 (1H, *s*), 6 9–7 3 (3H, complex, overlapped with the solvent signal), MS *m/z* (rel int) 336 $[M]^+$ (23), 294 (100), 252 (8), 234 (7), 217 (8), MS *m/z* 336 1208 $[M]^+$ (calc for $C_{17}H_{20}O_7$ 336 1208)

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IRIDOID GLUCOSIDES FROM *MELAMPYRUM*

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Key Word Index—*Melampyrum arvense*, *M cristatum*, Scrophulariaceae, iridoid glucosides, gardoside methyl ester, mussaenosidic acid, aucubin, 8-epiloganin, mussaenoside, melampyroside

Abstract—*Melampyrum arvense* and *M cristatum* contain, besides aucubin, 8-epiloganin and melampyroside, a new natural iridoid glucoside gardoside methyl ester In addition, *M arvense* contains mussaenoside and *M cristatum* mussaenosidic acid, another novel iridoid glucoside

INTRODUCTION

Chromatographic investigations [1, 2] have shown that aucubin (1) and catalpol, as well as esters of these compounds, are common in the genus *Melampyrum* However, only two species have so far been investigated in detail From *M silvaticum* L, aucubin (1) and melampyroside (3) [3] and, more recently, mussaenoside (2), globularifolin, catalpol and monomelittoside were isolated [4] From *M laxum* Miq, 1, 2 and 3 have been obtained [5] In the present work we give details of the isolation and characterization of iridoid glucosides from *M arvense* L and *M cristatum* L

RESULTS AND DISCUSSION

Five iridoid glucosides were isolated from *M arvense*, namely aucubin (1), mussaenoside (2), melampyroside (3), 8-epiloganin (4) and gardoside methyl ester (5) Compounds 1–4 were identified by their ^1H and ^{13}C NMR spectra [6–8], while the structure of 5 was deduced in the following way Its ^{13}C NMR spectrum displayed signals corresponding to an iridoid glucoside substituted at C-4 with a methoxycarbonyl group Additional signals at δ151 2 (*s*) and 113 9 (*t*) proved the presence of an exocyclic double bond, other structural features included a carbon atom substituted with a