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A NOVEL METHOD OF ISOLATION OF PHYTOECDYSONES FROM KALADANA SEEDS

LUIGI CANONICA, BRUNO DANIELI, GIORGIO FERRARI,* JIRI KREPINSKY* and IRENE WEISZ-VINCZE†

Istituto di Chimica Organica dell'Università, 20133 Milano, Italy; * Simes Research Laboratories, 20161 Milano Italy; and † Institute of Organic Chemistry, University of Szeged, Hungary

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Key Word Index—*Calonyction* sp.; Convolvulaceae; kaladana; crustecdysone; makisterone A; muristerone A; kaladasterone; calonysterone; methyl 3,4-dihydroxycinnamate.

Abstract—A new simplified technique is described for the isolation and separation of phytoecdysones from kaladana seeds. Using this method ecdysone, crustecdysone, muristerone A, kaladasterone, calonysterone and makisterone A were obtained. In addition methyl 3,4-dihydroxycinnamate was found in the seed.

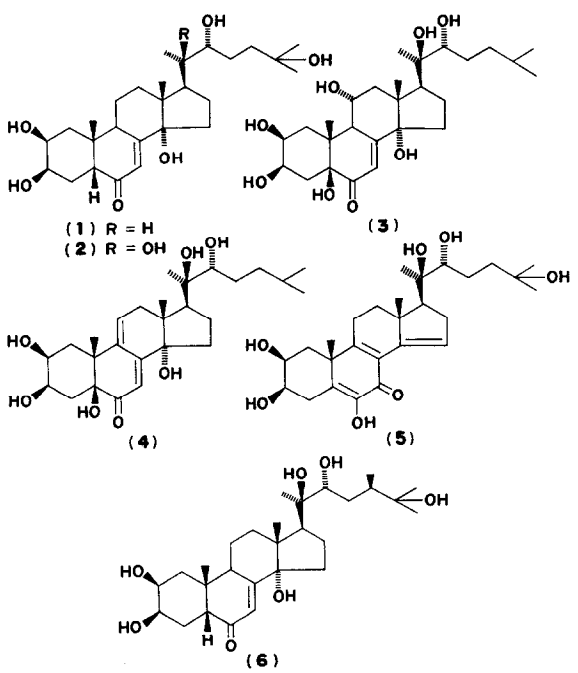
INTRODUCTION

Kaladana is the local name for the seeds of a plant in the Convolvulaceae which grows at about 1300–2000 m on the southern slopes of the Himalayas and it was used for centuries in indigenous medicine for its purgative and febrifuge effects [1–3]. Considerable confusion prevails regarding the classification of the plant; it is sometimes referred to as either *Ipomoea muricata* or *I. hederacea*. It differs quite clearly morphologically, as well as chemotaxonomically, from both [1–3], and this led to its classification as a new species in the genus *Calonyction* (Choisy) Hallier f., section Ipomoea, Convolvulaceae.* Kaladana seeds of various origins contain considerable amounts of lysergol and chanoclavine [2,3]. We found that the drug (kaladana seeds) contains not only these alkaloids but also considerable amounts of several polyhydroxy steroids with ecdysone-like structures [4]. This finding prompted us to undertake a detailed study which resulted in the isolation of six phytoecdysones, three having new structures [5–7]. We now report the procedure employed for the isolation of these compounds since it is suitable for large-scale work and is different from methods so far reported [4].

* We could not find any of the phytoecdysones described in this paper in authentic specimens of seeds of *I. muricata* and *I. hederacea*.

RESULTS AND DISCUSSION

Our separation procedure, which allows the isolation of relatively pure phytoecdysones, is based on the different solubilities of the various compounds in MeOH and H_2O. It is simpler than previous isolation techniques [4] and utilizes chromatographic separations to only a limited extent. Full details are given in the Experimental. The phytoec-



dysones obtained in this way were generally of 90–95% purity and the final purification was usually achieved by chromatography on silica gel and/or repeated crystallization. In this way we obtained ecdysone (**1**), crustecdysone (**2**), muristerone A (**3**), kaladasterone (**4**), and calonysterone (**5**). In the case of makisterone A (**6**), the 2,3,22-triacetate was prepared and, after purification by chromatography on silica gel and subsequent saponification of the fractions containing pure triacetate, makisterone A was obtained in a high state of purity. Compounds **1**, **2** and **6**, were identified by comparison of the physico–chemical and spectral data of these compounds and their derivatives, with the data reported in the lit. [4]; the determination of the structures of compounds **3**, **4** and **5** is described elsewhere [5–7]. The somewhat larger quantities of these compounds at our disposal allowed us to purify them thoroughly which in some cases led to better defined melting points and optical rotations.

A small quantity of methyl 3,4-dihydroxycinnamate was also isolated and identified by direct comparison with an authentic sample.

EXPERIMENTAL

M.ps were determined in an open capillary and are uncorr. NMR spectra are expressed in δ (ppm) from TMS as internal standard. TLC was carried out on silica gel GF_{254} (Merck) using CH_2Cl_2–MeOH–C_6H_6 (5:1:1) and 3% vanillin in EtOH + 0·5% conc. H_2SO_4 as spray reagent.

Isolation of compounds. Finely ground seeds of kaladana (40 kg) were defatted by stirring three times with light petrol. (30–60°; 70 l.). The defatted material was extracted 3× with 120 l. $CHCl_3$–MeOH–NH_4OH (9:0·9:0·1) for 12 hr, with stirring at room temp., and 3× with 100 l. of $CHCl_3$; the combined extracts were then evaporated at 40° to 1/10 of the original volume. The solid material which separated out after 4–5 days standing in a refrigerator was collected, washed with $CHCl_3$, and dried. The mother liquors, after concentration, deposited more solid after storage in the refrigerator, this was also collected, washed with $CHCl_3$, and dried. The combined solids (fraction A) totalled 281 g. The mother liquors contained alkaloids (75 g) and more compounds belonging to fraction A; the latter were isolated by direct extraction with H_2O and the residue, after evaporation to dryness (55 g), was combined with fraction A, (total 336 g).

Fraction A was triturated 4× with H_2O (1500 ml) at 25° for 1 hr. The insoluble part (90 g) contained alkaloids, while the dissolved material after evaporation to dryness (fraction B, 250 g) consisted mainly of phytoecdysones. Fraction B was triturated 4× with H_2O (1000 ml) at 25° for 1 hr. The combined filtrates after addition of MeOH (48) were concentrated under reduced pressure at 35° to *ca* 1/5 volume, allowed to crystallize in an ice-bath, and the crystalline fraction C (50 g) was collected. Fraction C after being dissolved in MeOH + 1% H_2O deposited crystals of reasonably pure makisterone A (**6**, 10 g), while crustecdysone (**2**) remained in the mother liquors and was obtained by evaporation of the solvent (residue R_1).

The mother liquors after precipitation of the crystalline fraction C were evaporated to dryness (110 g) and subjected to chromatography on a silica gel column (Merck, 1000 g) and fractions eluted with EtOAc were collected and combined. After evaporation to dryness they were redissolved in EtOAc and allowed to crystallize giving muristerone A (**3**, 10 g). The later fractions eluted with EtOAc + 10% MeOH yielded crustecdysone (**2**, 20 g). Repeated chromatography of the mother liquors from the muristerone A crystallisation on SiO_2 and elution with EtOAc gave 3,4-dihydroxycinnamate (**7**, 1·5 g), calonysterone (**5**, 0·3 g) and kaladasterone (**4**, 0·2 g).

The water insoluble part of fraction B, still wet after treatment with H_2O, was triturated with MeOH (200 ml) at 50°. The insoluble portion contained more alkaloids (9·5 g) while the soluble fraction D, after evaporation to dryness below 35°, was repeatedly crystallized from H_2O yielding ecdysone (**1**, 5 g) as a solid and leaving crustecdysone (**2**) in the mother liquors from which it was obtained by evaporation to dryness and crystallization (residue R_2). The two portions (R_1 and R_2) of crustecdysone (**2**) were combined and crystallized from Me_2CO to give relatively pure crustecdysone (**2**, 60 g).

Ecdysone (**1**) $C_{27}H_{44}O_6$, m.p. 240–241° (MeOH–Me_2CO, 1:2), $[\alpha]_D^{20}$ +67·8° (*c* 1, MeOH), lit. [4] m.p. 242° $[\alpha]_D^{20}$ + 64·2° ± 2° (*c* 1, EtOH). UV λ_{max} (MeOH): 244 nm (ϵ 11800). IR: ν_{max}^{Nujol} 3600–3200, 1650 and 1615 cm^{-1}. NMR (C_5H_5N): 0·74 (*s*, C–18$\underline{H}_3$), 1·10 (*s*, C–19$\underline{H}_3$), 1·30 (*d*, *J* 6 Hz, C–21$\underline{H}_3$), 1·40 (*s*, C–26$\underline{H}_3$C–27$\underline{H}_3$), 6·12 (*d*, *J* 2 Hz, C–7$\underline{H}$).

Ecdysone 2,3,22-*triacetate* was obtained as the monohydrate, m.p. 118–121°, $[\alpha]_D^{20}$ +54° (*c* 1, MeOH). (Found: C, 65·4; H, 8·5. $C_{33}H_{50}O_9 . H_2O$ requires: C, 65·1; H, 8·5%). NMR ($CDCl_3$): 0·67 (*s*, C–18$\underline{H}_3$), 0·94 (*d*, *J* 6 Hz, C–21$\underline{H}_3$), 1·03 (*s*, C–19$\underline{H}_3$), 1·21 and 1·23 (*s*, C–26$\underline{H}_3$ C–27$\underline{H}_3$), 1·99, 2·04, 2·11 (*s*, 3C$\underline{H}_3$COO–), 2·34 (*dd*, J_1 11, J_2 7 Hz, C–5$\underline{H}$), 3·12 (*ddd*, J_1 11, J_2 5, J_3 2·5 Hz, C–9$\underline{H}$), 4·86 (*m*, C–22$\underline{H}$), 5·06 (*ddd*, J_1 11, J_2 J_3 4 Hz, C–2$\underline{H}$), 5·34 (*m*, $W_{1/2}$ 5 Hz, C–3$\underline{H}$), 5·86 (*d*, *J* 2·5 Hz, C–7$\underline{H}$).

Ecdysone 2,3,22,25-*tetracetate* was obtained as the hemihydrate, m.p. 124–126°, $[\alpha]_D^{20}$ +65° (*c* 1, MeOH). (Found: C, 65·4; H, 8·2. $C_{35}H_{52}O_{10} . 1/2\ H_2O$ requires: C, 65·5, H, 8·3%). NMR ($CDCl_3$): 0·67 (*s*, C–18$\underline{H}_3$), 0·94 (*d*, *J* 6 Hz, C–21$\underline{H}_3$), 1·03 (*s*, C–19$\underline{H}_3$), 1·41 and 1·43 (*s*, C–26$\underline{H}_3$, C–27$\underline{H}_3$), 1·97, 1·99, 2·04, 2·10 (*s*, 4 C$\underline{H}_3$ COO–), 2·38 (*dd*, J_1 11, J_2 7 Hz, C–5$\underline{H}$), 3·12 (*ddd*, J_1 11, J_2 5, J_3 2·5 Hz, C–9$\underline{H}$), 4·86 (*m*, C–22$\underline{H}$), 5·06 (*ddd*, J_1 11, J_2 J_3 4 Hz, C–2$\underline{H}$), 5·34 (*m*, $W_{1/2}$ 5 Hz, C–3$\underline{H}$), 5·86 (*d*, *J* 2·5 Hz, C–7$\underline{H}$).

Crustecdysone (**2**), $C_{27}H_{44}O_7$, m.p. 238° (MeOH–Me_2CO, 1:2), (lit. [4] 234° or 237·5–239·5°), $[\alpha]_D^{20}$ +60·5° (*c* 1, MeOH), λ_{max} (MeOH) 244 nm (ϵ 12400). IR: ν_{max}^{Nujol} 3600–3200, 1650 and 1610 cm^{-1}. NMR (C_5D_5N): 1·06 (*s*, C–19$\underline{H}_3$), 1·20 (*s*, C–18$\underline{H}_3$), 1·33 and 1·35 (*s*, C–26$\underline{H}_3$, C–27$\underline{H}_3$), 1·55 (*s*, C–21$\underline{H}_3$), 6·13 (*d*, *J* 2·5 Hz, C–7$\underline{H}$). NMR (DMSO-d_6): 0·78 (*s*, C–18$\underline{H}_3$), 0·85 (*s*, C–19$\underline{H}_3$), 1·08 (*s*, C–21$\underline{H}_3$), 1·10 (*s*, C–26$\underline{H}_3$, C–27$\underline{H}_3$), 5·65 (*d*, *J* 2 Hz, C–7$\underline{H}$).

Crustecdysone 2,3,22-*triacetate* was obtained as the monohydrate, m.p. 155–157°, $[\alpha]_D^{20}$ +58·8, (lit. [4] 198·5–199°). (Found: C, 63·4; H, 8·4, $C_{33}H_{50}O_{10} . H_2O$ requires: C, 63·5, H, 8·3%). NMR ($CDCl_3$): 0·82 (*s*, C–18$\underline{H}_3$), 1·00 (*s*, C–19$\underline{H}_3$), 1·18 (*s*, C–21$\underline{H}_3$), 1·20 and 1·22 (*s*, C–26$\underline{H}_3$, C–27$\underline{H}_3$), 1·97 (*s*, C$\underline{H}_3$COO–), 2·09 (*s*, 2C$\underline{H}_3$COO), 3·12 (*ddd*, J_1 11, J_2 5, J_3 2·5 Hz, C–9$\underline{H}$), 4·82 (*m*, C–22$\underline{H}$), 5·08 (*ddd*, J_1 11, J_2 J_3 4 Hz, C–2$\underline{H}$), 5·31 (*m*, $W_{1/2}$ 7 Hz, C–3$\underline{H}$), 5·86 *d J* 2·5 Hz, C–7$\underline{H}$).

Crustecdysone 2,3,22,25 *tetracetate* was obtained as the hemihydrate, m.p. 200° (lit. [4] 200·5 201°), $[\alpha]_D^{20}$ +60° (*c* 1, MeOH). NMR ($CDCl_3$): 0·87 (*s*, C–18$\underline{H}_3$), 1·04 (*s*, C–19$\underline{H}_3$), 1·26 (*s*, C–21$\underline{H}_3$), 1·42 and 1·44 (*s*, C–26$\underline{H}_3$, C–27$\underline{H}_3$), 1·99 (*s*, C$\underline{H}_3$COO–), 2·01 (*s*, C$\underline{H}_3$COO–), 2·11 (*s*, 2 C$\underline{H}_3$COO–), 3·14 (*ddd*, J_1 11 J_2 5 J_3 2·5 Hz, C–9$\underline{H}$), 4·82 (*m*, C–22$\underline{H}$), 5·08 (*s*, *ddd*,

J_1, J_2 J_3 4 Hz, C–2H), 5·32 (*m*, $W_{1/2}$ 7 Hz, C–3H), 5·86 (*d*, *J* 2·5 Hz, C–7H).

Makisterone A (**6**), $C_{28}H_{46}O_7$ was isolated via the 2,3,22-triacetate (4·1 g), m.p. 236°–238° (MeOH), (lit. [8] 210–213°), $[\alpha]_D^{20}$ +74·3 (*c* 1, MeOH). (Found: C, 65·4; H, 8·4. $C_{34}H_{52}O_{10}$ requires: C, 65·8; H, 8·4%). NMR ($CDCl_3$): 0·87 (*s*, C–18H$_3$), 0·94 (*d*, *J* 7 Hz, C–28H$_3$), 1·03 (*s*, C–19H$_3$), 1·16 (*s*, C–21H$_3$), 1·19 and 1·26 (*s*, C–26H$_3$ and C–27H$_3$), 2·00 (*s*, CH$_3$COO–), 2·11 (*s*, 2CH$_3$COO–), 4·98 (*m*, C–22H), 5·06 (*ddd*, J_1, 11, J_2 J_3 4 Hz, C–2H), 5·32 (*m*, $W_{1/2}$ 7 Hz, C–3H), 5·86 (*d*, *J* 2·5 Hz, C–7H). Hydrolysis of the triacetate gave makisterone A (1·4 g), m.p. 286°–287° (lit. [4] 263–265°), $[\alpha]_D^{20}$ +83·3° (*c* 1, MeOH). (Found: C, 67·7; H, 9·3. $C_{28}H_{46}O_7$ requires: C, 67·6; H, 9·25%). λ_{max} (MeOH) 244 nm (ε 12400). IR: ν_{max}^{Nujol} 3600–3200, 1650 and 1610 cm^{-1}. NMR (C_5D_5N): 1·06 (*s*, C–19H$_3$), 1·07 (*d*, *J* 7 Hz, C–28H$_3$), 1·24 (*s*, C–18H$_3$), 1·32 (*s*, C–26H$_3$ and C–27H$_3$), 1·57 (*s*, C–21H$_3$), 6·20 (*d*, *J* 2 Hz, C–7H).

Makisterone A 2,3,22,25-tetracetate was obtained as the hemihydrate, m.p. 164°–166°, $[\alpha]_D^{20}$ +74° (*c* 1, MeOH). (Found: C, 64·2; H, 8·2. $C_{36}H_{54}O_{11}\cdot\frac{1}{2}H_2O$ requires: C, 64·4; H, 8·2%). NMR ($CDCl_3$): 0·87 (*s*, C–18H$_3$), 0·93 (*d*, *J* 7 Hz), C–28H$_3$), 1·03 (*s*, C–19H$_3$), 1·35 and 1·44 (*s*, C–26H$_3$, C–27H$_3$), 1·97 (*s*, CH$_3$COO–), 2·00 (*s*, CH$_3$COO–), 2·11 (*s*, 2CH$_3$COO–), 3·12 (*ddd*, J_1 11, J_2 5 J_3 2·5 Hz, C–9H), 4·94 (*m*, C–22H), 5·06 (*ddd*, J_1 11, J_2 = J_3 4 Hz, C–2H, 5·32 (*m*, $W_{1/2}$ 7 Hz, C–3H), 5·86 (*d*, *J* 2·5 Hz, C–7H).

Muristerone A (**3**), $C_{27}H_{44}O_8$, m.p. 238–244° (MeOH), $[\alpha]_D^{20}$ +49·6° (*c* 1, pyridine) was obtained by chromatography of slightly impure compound (10 g) on deactivated silica gel (500 g) and elution with CH_2Cl_2 + 4% MeOH. λ_{max} (MeOH) 236 nm (ε 8900). IR ν_{max}^{KBr}: 3600–3100, 1660 and 1630 cm^{-1}. NMR (C_5D_5N): 0·82 (*d*, *J* 6 Hz, C–26H$_3$ and C–27H$_3$), 1·24 (*s*, C–18H$_3$), 1·38 (*s*, C–19H$_3$), 1·53 (*s*, C–21H$_3$), 6·28 (*d*, *J* 2·5 Hz, C–7H).

Kaladasterone (**4**), $C_{27}H_{42}O_7$, m.p. 242–243° (MeOH–Me_2CO, 1:2), $[\alpha]_D^{20}$ +79·3° (*c* 1, MeOH) was obtained by chromatography of slightly impure compound (1·5 g) on a column of deactivated silica gel (1000 g) and elution with CH_2Cl_2 + 5% MeOH. λ_{max} (MeOH) 298 nm (ε 10800). IR ν_{max}^{KBr} 3600–3200, 1652 and 1605 cm^{-1}. NMR (DMSO-d_6): 0·73 (*s*, C–18H$_3$), 0·86 (*d*, *J* 7 Hz, C–26H$_3$ and C–27H$_3$), 0·96 (*s*, C–19H$_3$), 1·06 (*s*, C–21H$_3$), 5·65 (*s*, C–7H), 6·18 (*m*, $W_{1/2}$ 10 Hz, C–11H).

Calonysterone (**5**), $C_{27}H_{40}O_7$, m.p. 253–254° (Me_2CO), $[\alpha]_D^{20}$ +76·8 (*c* 1, MeOH), λ_{max} 222, 244 and 294 nm (ε 20700, 13500 and 7850). IR ν_{max}^{KBr} 3380, 3350, 1638, 1620 and 845 cm^{-1}. NMR (DMSO-d_6): 1·00 (*s*, C–18H$_3$), 1·05 and 1·07 (*s*, C–26H$_3$, C–27H$_3$), 1·16 (*s*, C–21H$_3$), 1·41 (*s*, C–19H$_3$), 6·78 (*s*, C–15H).

Methyl 3,4-dihydroxycinnamate, $C_{10}H_{10}O_4$, m.p. 159–160° (MeOH–H_2O), (lit. [9] 159–160°). NMR (C_5D_5N): 3·68 (*s*, –OCH$_3$), 6·32 (*d*, *J* 17 Hz, H–C=C–(H)–COOMe), 7·69 (*d*, *J* 17 Hz, H–C=C–(H)–COOMe), 6·96 (*bs*, 2 aromatic H, 7·27 (*bs*, aromatic H, 9·70 (*bs*, 2–OH).

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