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IGUESTERIN, A NEW QUINONOID TRITERPENE FROM *CATHA CASSINOIDES*

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Key Word Index—*Catha cassinoides*; Celastraceae; peralillo; sitosterol; β-amyrin; quinonoid triterpenes; iguesterin; celastrol; pristimerin; tingenone; PMR.

Abstract—β-Amyrin, sitosterol, celastrol, pristimerin, tingenone and a new hydroxymethylene quinonoid dinor-triterpene iguesterine were isolated from the root bark of *Catha cassinoides*. The structure of iguesterine was determined spectroscopically and by synthesis from tingenone. Tentative assignments are given for the chemical shifts of the methyl groups of iguesterin, tingenone and some of their derivatives.

INTRODUCTION

In a short communication [1] we reported the isolation of a new hydroxymethylene quinonoid dinor-triterpene iguesterin from the root bark of *Catha cassinoides*. On the basis of spectral data it was assigned structure **1**, which is now confirmed. In addition to **1**, β-amyrin and pristimerin (**2b**) cited previously [1], we have also isolated sitosterol, celastrol (**2a**) and tingenone (**3**), an antitumour substance whose structure has been elucidated recently [2, 3].

Based on a detailed study of the PMR spectra, tentative assignments for the chemical shifts of the methyl groups in **1**, **3** and some of their derivatives (Table 1) are given, taking into account the long range effects of the C-20 double bond and the C-21 carbonyl group by comparison with 21-desoxydiacetyldihydrotingenone (**7**).

RESULTS AND DISCUSSION

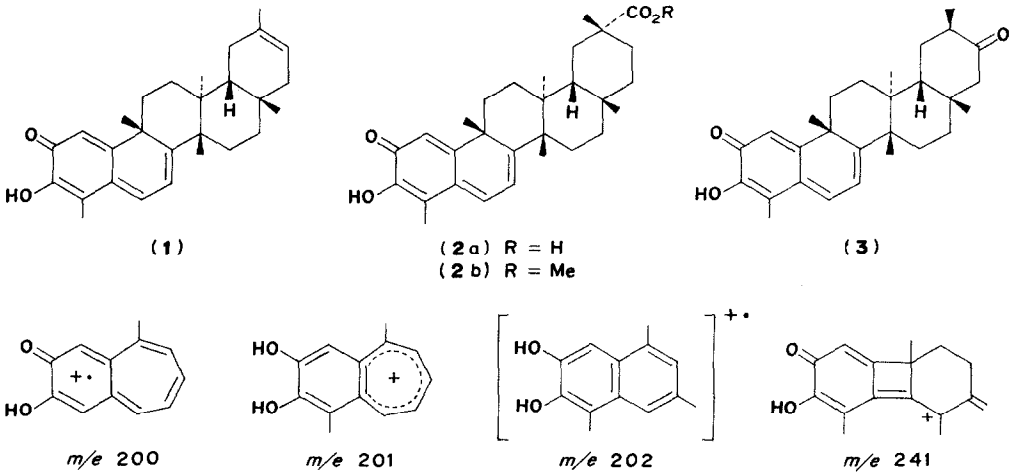
Iguesterin (**1**) ($C_{28}H_{36}O_2$, high resolution MS), a red–orange pigment which would not crystallize,

Table 1. PMR spectral data for iguesterin, tingenone and related compounds*

Compound	C-1	C-6	C-7	C-21	C-4 Me	C-9 Me	C-13 Me	C-14 Me	C-17 Me	C-20 Me	OAc
1	3·44	2·97	3·66	4·76	7·79	8·52	9·49	8·68	9·00	8·39	—
	s	AB(7)	AB(7)	*m*[11]	*s*	*s*	*s*	*s*	*s*	*d*(2)	
3	3·45	2·98	3·65	6·96 *m*[5]†	7·79	8·50	9·00 or	8·67	9·04 or	9·03	—
	s	AB(7)	AB(7)	7·20 *m*[5]	*s*	*s*	9·04 *s*	*s*	9·00 *s*	*d*	
4	2·96	6·78	4·20	6·93 *m*[6]†	7·94	8·62	9·00 or	8·72	9·03 or	9·04	7·71 *s*
	s	*m*[14]	*m*[11]	7·19 *m*[6]	*s*	*s*	9·03 *s*	*s*	9·00 *s*	*d*	7·75 *s*
5a	2·97	6·78	4·21	6·16	7·93	8·63	9·18	8·74	8·76	9·13	7·70 *s*
	s	*m*[14]	*m*[11]	*m*[9]	*s*	*s*	*s*	*s*	*s*	*d*	7·74 *s*
5b	3·24	6·80	4·22	6·13	7·84	8·68	9·18	8·73	8·77	9·10	—
	s	*m*[14]	*m*[11]	*m*[9]	*s*	*s*	*s*	*s*	*s*	*d*	
6	2·98	6·80	4·26	4·78	7·95	8·65	9·46	8·73	9·04	8·42	7·73 *s*
	s	*m*[14]	*m*[11]	*m*[11]	*s*	*s*	*s*	*s*	*s*	*d*(2)	7·77 *s*
7	2·98	6·80	4·25		7·94	8·64	9·18	8·78	8·97	9·09	7·72 *s*
	s	*m*[14]	*m*[12]		*s*	*s*	*s*	*s*	*s*	*d*	7·76 *s*

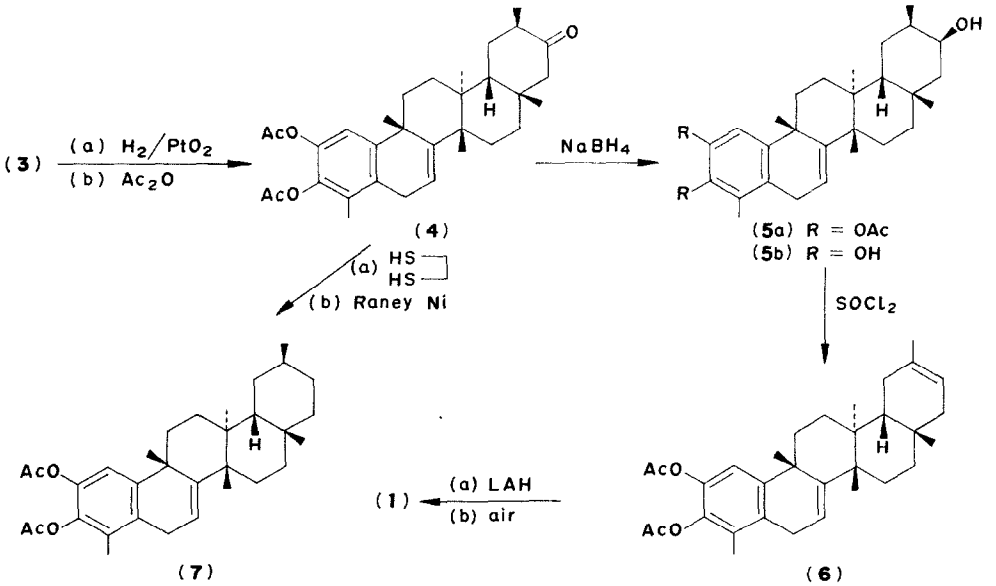
* Determined at 60 MHz in $CDCl_3$, chemical shifts are given in τ, coupling constants *J* in parentheses, $W_{1/2}$ in brackets (both in Hz).

† C-20.



was separated from β-amyrin by PLC on silica gel. The similarity of its chromophore with that of pristimerin (**2b**) and tingenone (**3**) was easily established by comparison of their PMR, UV, visible and IR spectral data. Thus, in the PMR spectrum of iguesterine an AB system at τ 2·97 and 3·66 (J 7 Hz) corresponded to the C-6 and C-7 protons respectively, as confirmed by spin decoupling, and two singlets at τ 3·44 and 7·79 to the C-1 and C-4 methyl protons (Table 1) [2–5]. In the IR spectrum characteristic absorptions of the C-2 carbonyl (1595 cm^{-1}) and the intramolecularly associated hydroxyl at C-3 (3380 cm^{-1}) were observed, similar to those of **2b** and **3**. Their UV and visible spectra were superimposable. Moreover, the behaviour of the chromophore in the presence of reducing agents such as H_2 and $NaBH_4$ was the same for all three compounds: they were rapidly decoloured, but oxidized again by air. This resemblance, as well as the close relationship of the carbon skeletons, was also confirmed by comparison of their MS which displayed characteristic fragments at *m/e* 200, 201, 202 and 241 that originated by cleavage between the rings *B/C* and *C/D* [2].

In the lowfield region the PMR spectrum of iguesterin (**1**) differed from that of **3** only by a multiplet at τ 4·76 (1 H, $W_{1/2}$ = 11 Hz) indicating the presence of a trisubstituted non-conjugated double bond which could only be located at C-20, the signal for the C-20 methyl group appearing as a



doublet at τ 8·39 (J 2 Hz). A singlet at τ 9·49, absent from the spectra of tingenone (**3**) and its derivatives **4**, **5** and **7**, corresponded to the C-13 methyl group which lies below the plane of the double bond at C-20. This strong shielding due to the long range effect of the double bond [6] was also observed in the spectra taken in CCl_4 and C_6D_6 (τ 9·49 and 9·51 respectively) as well as in the spectra of compound **6** (τ 9·46 in $CDCl_3$ and CCl_4) and is only in accordance with the stereochemistry of the rings *C*, *D* and *E* indicated in structure **1**. This structure, proposed on the basis of both spectroscopic and phytochemical evidence, was confirmed by obtaining iguesterin from tingenone (**3**) by the route shown in the Scheme. Hydrogenation of **3** over PtO_2 and subsequent acetylation in a N_2 atmosphere gave the diacetyl–dihydro derivative **4**, whose UV, IR and PMR data were consistent with those reported [3]. Reduction of the carbonyl group at C-21 with $NaBH_4$ in EtOH yielded a mixture of **5a**, unstable triol **5b** (ratio *ca* 2:1) and starting material which was separated by column chromatography.* The fact that in the PMR spectrum of **5a** ($C_{32}H_{44}O_5$, high resolution MS) the proton geminal to the hydroxyl was observed as a narrow multiplet at τ 6·16 ($W_{1/2}$ 9 Hz) indicated that the C-21 hydroxyl was axial. Dehydration of **5a** with $SOCl_2$ at room temperature gave compound **6** ($C_{32}H_{42}O_4$, high resolution MS), which lacked hydroxyl absorption in the IR spectrum. In the PMR spectrum the C-20 methyl protons appeared as a doublet at τ 8·42 (J 2 Hz) and the vinyl proton at C-21 as a multiplet at 4·78 ($W_{1/2}$ 11 Hz), the same as for iguesterin. Deacetylation of **6** with $LiAlH_4$ at reflux for a short time followed by stirring at room temperature yielded the corresponding unstable diol which on oxidation by air gave iguesterin (**1**), identical with the natural pigment. Biogenetically, iguesterin may originate from celastrol (**2a**) by oxidative decarboxylation.

EXPERIMENTAL

Mps, determined on a Kofler block, are uncorr. Optical rotations were measured in $CHCl_3$ and UV/VIS spectra in EtOH. Dry column chromatography was performed on Si gel (0·063–0·20 mm) and PLC on Si gel $PF_{254+366}$. The spray reagent for TLC was H_2SO_4–HOAc–H_2O (1:20:4).

Isolation of the triterpenoids. The root bark (1·5 kg) of the plant, collected near Igueste de Candelaria (Tenerife) in December 1973, was finely cut and extracted 3× with boiling Et_2O. The red extract (30 g) was chromatographed on a dry column (C_6H_6; C_6H_6–EtOAc), yielding pristimerin (**2b**; 0·10%), sitosterol (0·01%), tingenone (**3**; 0·12%), celastrol (**2a**; 0·004%, very impure), and a mixture of β-amyrin (0·03%) and iguesterin (**1**; 0·02%) which was separated by repeated PLC (thickness 1 mm; 1 elution; C_6H_6–EtOAc, 9:1).

Iguesterin (**1**), amorphous, $[\alpha]_D$ −99° (*c*, 0·264). MS: *m/e* 404·2726 (M^+, 23%; $C_{28}H_{36}O_2$ requires 404·2715), 390 (M^+ − Me + H, 4%), 389 (M^+ − Me, 3%), 241 (13%), 202 (100%), 201 (50%), 200 (20%). IR ν_{max}^{CS}: 3380 (OH), ν_{max}^{KBr} 1595 cm^{-1} (C=O). UV λ_{max} (log ϵ): 254 sh (4·02), 420 nm (4·07). PMR ($CDCl_3$): Table 1; (CCl_4): τ 3·16 (1H, AB, J 7 Hz, C-6), 3·64 (1H, *s*, C-1), 3·79 (1H, AB, J 7 Hz, C-7), 4·80 (1H, *m*, $W_{1/2}$ 10 Hz, C-21), 7·87 (3H, *s*, C-4 Me), 8·41 (3H, *d*, J 2 Hz, C-20 Me), 8·56 (3H, *s*, C-9 Me), 8·70 (3H, *s*, C-14 Me), 9·03 (3H, *s*, C-17 Me), 9·52 (3H, *s*, C-13 Me); (C_6D_6): τ 3·40 (1H, *s*, C-1), 3·46 (1H, AB, J 7 Hz, C-6), 4·09 (1H, AB, J 7 Hz, C-7), 4·75 (1H, *m*, $W_{1/2}$ 11 Hz, C-21), 7·91 (3H, *s*, C-4 Me), 8·38 (3H, *d*, J 2 Hz, C-20 Me), 8·78 (3H, *s*, C-9 Me), 8·92 (3H, *s*, C-14 Me), 9·02 (3H, *s*, C-17 Me), 9·51 (3H, *s*, C-13 Me).

Celastrol (**2a**). Methylation with CH_2N_2 and purification by dry column chromatography (C_6H_6–EtOAc, 19:1) gave **2b** (60 mg), identical with the natural compound (mmp, TLC, IR, UV, VIS, PMR).

Pristimerin (**2b**), mp 214–217° (MeOH). MS: *m/e* 464 (M^+, 18%; calc. for $C_{30}H_{40}O_4$: 464), 450 (M^+ − Me + H, 5%), 241 (11%), 203 (100%), 202 (74%), 201 (35%), 200 (10%). IR ν_{max}^{KBr}: 3370 (OH), 1730 (ester), 1597 cm^{-1} (C=O). UV λ_{max} (log ϵ): 253 sh (4·03), 420 nm (4·10). PMR ($CDCl_3$): τ 2·98 (1H, AB, J 7 Hz, C-6), 3·44 (1H, *s*, C-1), 3·64 (1H, AB, J 7 Hz, C-7), 6·43 (3H, *s*, CO_2Me), 7·79 (3H, *s*, C-4 Me), 8·55 (3H, *s*, C-9 Me), 8·74, 8·83, 8·90, 9·47 (each 3H, *s*). Identical with an authentic sample (mmp, TLC, IR, UV, VIS, PMR).

Tingenone (**3**), mp unstable [7]. MS: *m/e* 420·2672 (M^+, 40%; calc. for $C_{28}H_{36}O_3$: 420·2664), 406 (M^+ − Me + H, 4%), 405 (M^+ − Me, 5%), 241 (13%), 202 (100%), 201 (46%), 200 (26%), IR ν_{max}^{KBr}: 3350 (OH), 1705 (C_{21}=O), 1595 cm^{-1} (C_2=O). UV λ_{max} (log ϵ): 254 sh (3·89), 420 nm (3·96). PMR: Table 1. Identical with an authentic sample (TLC, IR, UV, VIS, PMR).

Diacetyl-dihydrotingenone (**4**). Tingenone (**3**; 400 mg) in MeOH (97 ml) was hydrogenated over PtO_2 (70 mg) at room temp. and atm. pres. Acetylation of the product with Ac_2O–C_6H_5N in a N_2 atm. and usual work-up followed by dry column chromatography (C_6H_6–EtOAc, 19:1) gave **4** (288 mg), mp 208–210° (MeOH–H_2O). (Found: C, 76·42; H, 8·60. Calc. for $C_{32}H_{42}O_5$: C, 75·86; H, 8·36%.) IR ν_{max}^{KBr}: 1770 (OAc), 1700 cm^{-1} (C=O). UV λ_{max} (log ϵ): 266 (3·10), 274 nm (3·09). PMR: Table 1.

Reduction of **4**. To a soln of **4** (250 mg) in EtOH (40 ml) $NaBH_4$ (200 mg) in EtOH (20 ml) was added and the mixture stirred at room temp. for 1·5 hr. Standard work-up followed by dry column chromatography (C_6H_6–EtOAc, 22:3) gave starting material (33 mg), unstable triol **5b** (60 mg; PMR: Table 1) and **5a** (106 mg), m.p. 217–221° (MeOH), $[\alpha]_D$ −12° (*c*, 0·026). MS: *m/e* 508 (M^+, 20%; $C_{32}H_{44}O_5$ requires 508), 493·2951 (M^+ − Me, 72%; $C_{31}H_{41}O_5$ requires 493·2954), 475 (M^+ − Me − H_2O, 55%), 466 (M^+ − C_2H_2O, 10%), 451 (M^+ − Me − C_2H_2O, 20%), 285 (28%), 271 (49%), 229 (100%), 187 (100%). IR ν_{max}^{KBr}: 3560 (OH), 1765, 1750 cm^{-1} (OAc). UV λ_{max} (log ϵ): 266 (3·03), 275 nm (3·03). PMR: Table 1.

* Small quantities of the possible C-21-epimer of **5a** were also formed but not isolated. Attempts to diminish the amount of triol **5b** by reducing the quantity of $NaBH_4$, the reaction time or the polarity of the medium were unsuccessful and gave appreciable amounts of starting material.

Diacetyl-dihydroiguesterin (**6**). To **5a** (100 mg) in dry C_6H_5N (20 ml) $SOCl_2$ (0·5 ml) was added at 0° and the soln kept at room temp. for 1 hr. Usual work-up and purification by PLC (thickness 0·5 mm; C_6H_6 EtOAc, 47:3) gave **6** (67 mg), m.p. 184–185° (MeOH), $[\alpha]_D$ −27° (*c*, 0·228). MS: *m/e* 490 (M^+, 13%; $C_{32}H_{42}O_4$ requires 490), 475·2844 (M^+ − Me, 73%; $C_{31}H_{39}O_4$ requires 475·2848), 448 (M^+ − C_2H_2O, 4%), 433 (M^+ − Me − C_2H_2O, 14%), 285 (9%), 271 (33%), 229 (47%), 287 (41%), IR $\nu_{max}^{CS_2}$: 1765 cm^{-1} (OAc). UV λ_{max} (log ε): 253 (3·19), 266 (3·03), 275 nm (3·01). PMR: Table 1.

Iguesterin (**1**) *from* **6**. To a soln of **6** (55 mg) in dry Et_2O (20 ml) a suspension of $LiAlH_4$ (10 mg) in Et_2O (5 ml) was added under stirring and the mixture refluxed for 5 min, after which it was stirred until it reached room temp. (15 min). After destroying excess $LiAlH_4$ first with MeOH and then with H_2O the reaction product, which consisted of the diol of **6** and traces of **1** (formed during the standard work-up) was treated as usual and finally dissolved in $CHCl_3$ (50 ml). Acid-free air ($NaHCO_3$ trap) was passed through the soln for 80 hr. PLC (thickness 0·5 mm; C_6H_6–EtOAc, 93:7) of the extract yielded **1** (18 mg) which was identical to the natural pigment (TLC, IR, UV, VIS, PMR).

21-*Desoxy-diacetyldihydrotingenone* (**7**). Diacetyl-dihydrotingenone (**4**; 140 mg) was treated with ethanedithiol (3 ml) and 70% $HClO_4$ (2 drops) at room temp. for 30 min. After diluting with MeOH and usual work-up dry column chromatography (C_6H_6–EtOAc, 93:7) gave the 21-thioketal derivative of **4** [95 mg; PMR: τ 6·79 (4H, *s*, SH)] which was dissolved in EtOH (30 ml) and refluxed with Raney Ni W-7 (1 g; 3 additional washings with H_2O) for 3·5 hr. The reaction product was purified by dry column chromatography (C_6H_6–EtOAc, 19:1) to give **7** (35 mg), mp 189–191° (MeOH–H_2O). MS: *m/e* 492 (M^+, 25%; $C_{32}H_{44}O_4$ requires 492), 477 (M^+ − Me, 68%), 450 (M^+ − C_2H_2O, 18%), 435 (M^+ − Me − C_2H_2O, 20%), 285 (39%), 271 (75%), 229 (96%), 187 (75%). IR ν_{max}^{KBr}: 1765 cm^{-1} (OAc). PMR: Table 1.

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