

# Crotasteroiridocin: A New Steroid-iridoid from *Crotalaria emarginella* and its *anti*-Inflammatory Activity

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The aerial parts of *Crotalaria emarginella* Vatke (Leguminosae) have afforded a new steroid-iridoid, characterized as 1'-hydroxy-isoiridomyrmecynyl-1'-O- $\beta$ -3-O- $\alpha$ -stigmast-5-ene, designated as crotasteroiridocin (**1**), which shows a unique combination of iridoid and sterol units, found very rarely in nature. Furthermore, a rare iridoid glucoside namely, *bis*-desoxy-dihydro-monotropein (**2**) has also been isolated from the aerial parts of the plant. The structures of the isolated products were elucidated on the basis of spectral and chemical studies. The *anti*-inflammatory activity has also been screened, wherein compounds **1** and **2** have shown 25.92% and 28.39% activity respectively, with respect to phenyl butazone against carrageenan employing the rat paw method.

**Key words:** *Crotalaria emarginella* Vatke, Sterol-Iridoid, Crotasteroiridocin, Iridoid Glucoside

## Introduction

The plant *Crotalaria emarginella* Vatke (Leguminosae) is distributed in Tropical East Africa, Yemen and Saudi Arabia including Najad, Asir and the eastern region [1]. The plant is a woody-based bushy, leafy, much branched, perennial herb, and is used in traditional medicine for the treatment of scabies, impetigo, fever, and as a colic remedy. In India, the root is used as a haemoptysis remedy and against skin disease [2]. The plants of the genus *Crotalaria* are also used for cooling and purifying blood in impetigo and psoriasis [3]. A literature survey indicated that no phytochemical work has been done so far on the plant. We have isolated a new sterol-iridoid characterized as 1'-hydroxy-isoiridomyrmecynyl-1'-O- $\beta$ -3-O- $\alpha$ -stigmast-5-ene, and designated as crotasteroiridocin (**1**), which is a unique combination of iridoid and sterol units usually found very rarely in nature. In addition, a rare iridoid glucoside namely, *bis*-desoxy-dihydro-monotropein (**2**) from the aerial parts of the plant. The compounds **1** and **2** have shown 25.92% and 28.39% *anti*-inflammatory activity respectively compared to phenyl butazone against carrageenan in the rat paw method.

## Results and Discussion

### Part A: Phytochemical work

Crotasteroiridocin (**1**) was obtained as colorless needles exhibiting a molecular formula  $C_{39}H_{64}O_3$  as established on the basis of HR-MS (580.4850),  $^{13}C$  NMR and DEPT spectra. Its purity was checked by GC, which gave a single peak. It gave a positive Liebermann-Burchard test indicating it to be a sterol derivative. Its IR spectrum indicated the presence of an ether linkage ( $1139\text{ cm}^{-1}$ ), a lactone ring ( $1713\text{ cm}^{-1}$ ) and a double bond ( $1670, 978\text{ cm}^{-1}$ ). The  $^{13}C$  NMR and DEPT spectra [4] showed 39 carbon atoms for the molecule consisting of eight methyls, thirteen methylenes, eleven methines, two carbinolic, one olefinic, three quaternary and one carbonyl carbon atom (in total  $C_{39}H_{64}$ ). The assignments of proton and carbon atoms were made with the help of  $^1H$ - $^1H$ -COSY and HMQC experiments [5] starting with easily distinguishable olefinic and carbinolic protons. The carbinolic proton in the  $^1H$  NMR spectrum appeared at  $\delta_H = 3.87$  (dddd,  $J = 6.5, 4.5, 5.0, 6.5\text{ Hz}$ ,  $\delta_C = 78.8$ ) attributable to position-3 biogenetically, and on the basis of fragmentation pattern of its

Table 1. 1D- and 2-D NMR data of crotasteroiridocin (1).

Position	<sup>1</sup> H NMR*	<sup>13</sup> C NMR HMQC*	DEPT**	<sup>1</sup> H- <sup>1</sup> H COSY*	HMBC*** <sup>2</sup> J <sub>CH</sub>	<sup>3</sup> J <sub>CH</sub>
1a	0.90 <i>ddd</i> (16.0, 9.5, 5.3)	36.1 <i>t</i>	CH <sub>2</sub>	H-1b, H <sub>2</sub> -2	C-2, C-10	C-3, Me-19
1b	1.65 <i>ddd</i> (16.0, 8.5, 4.5)	—	—	H-1a, H <sub>2</sub> -1	C-2, C-10	C-3, Me-19
2a	1.40 <i>ddd</i> (15.0, 8.5, 4.5)	29.4 <i>t</i>	CH <sub>2</sub>	H-2b, H-3, H <sub>2</sub> -1	C-1, C-3	C-10, C-5
2b	1.78 <i>m</i>	—	—	H-2a, H-3, H <sub>2</sub> -1	C-1, C-3	C-10
3β	3.87 <i>dddd</i> (6.5, 4.5, 5.0, 6.5)	78.8 <i>d</i>	CH	H <sub>2</sub> -2, H <sub>2</sub> -4	C-2, C-4	C-1'
4a	2.02 <i>dd</i> (11.5, 12.5)	38.6 <i>t</i>	CH <sub>2</sub>	H-4b, H-3	C-3, C-5	C-6
4b	2.08 <i>ddd</i> (12.5, 6.5, 5.0)	—	—	H-4a, H-3	C-3, C-5	C-2
5	—	140.3 <i>s</i>	C	—	—	—
6	5.13 <i>t</i> (7.5)	122.1 <i>d</i>	CH	H <sub>2</sub> -7	C-5, C-7	C-10, C-8, C-4
7a	1.62 <i>m</i>	28.2 <i>t</i>	CH <sub>2</sub>	H-7b, H-6	C-6, C-8	C-5
7b	1.05 <i>m</i>	—	—	H-7a, H-8	C-6, C-8	C-9
8	0.93 <i>m</i>	56.1 <i>d</i>	CH	H-7a, H-9	C-9, C-7	C-6, C-10
9	0.70 <i>m</i>	49.7 <i>d</i>	CH	H-8, H <sub>2</sub> -11	C-8, C-10, C-11	C-7, Me-19
10	—	36.7 <i>s</i>	C	—	—	—
11a	1.25 <i>ddd</i> (15.0, 9.5, 4.0)	21.1 <i>t</i>	CH <sub>2</sub>	H-9, H-11b, H <sub>2</sub> -12	C-9, C-12	C-8, C-13
11b	1.28 <i>ddd</i> (15.0, 6.5, 5.0)	—	—	H-9, H-11a, H <sub>2</sub> -12	C-9, C-12	C-13
12a	1.78 <i>m</i>	31.6 <i>t</i>	CH <sub>2</sub>	H <sub>2</sub> -11	C-11,	C-13, Me-18, C-14
12b	1.81 <i>m</i>	—	—	H <sub>2</sub> -11	C-11	Me-18, C-14
13	—	42.3 <i>s</i>	C	—	—	—
14	0.84 <i>m</i>	56.7 <i>d</i>	CH	H-8, H <sub>2</sub> -15	C-8, C-15, C-13	C-16
15a	1.78 <i>m</i>	31.9 <i>t</i>	CH <sub>2</sub>	H <sub>2</sub> -16, H-14, H-15a	C-14, C-16	C-8, C-17
15b	1.80 <i>m</i>	—	—	H-14, H-15a, H <sub>2</sub> -16	C-14, C-17	C-8
16a	0.95 <i>ddd</i> (13.0, 6.5, 3.5)	39.7 <i>t</i>	CH <sub>2</sub>	H-16b, H-17, H <sub>2</sub> -15	C-15, C-17	C-14
16b	1.80 <i>m</i>	—	—	H-16a, H-17	C-15, C-17	C-13
17	2.09 <i>dd</i> (9.0, 8.5)	39.4 <i>d</i>	CH	H <sub>2</sub> -16, H-20	C-20, C-13	C-16
18	0.46 <i>s</i>	11.97 <i>q</i>	CH <sub>3</sub>	—	C-13	C-12, C-14, C-17
19	0.79 <i>s</i>	18.8 <i>q</i>	CH <sub>3</sub>	—	C-10	C-5, C-1, C-9
20	1.12 <i>m</i>	31.9 <i>d</i>	CH	H-17, Me-21, H <sub>2</sub> -22	Me-21, C-17, C-22	C-23
21	0.71 <i>d</i> (6.0)	18.7 <i>q</i>	Me	H-20	C-20	C-17, C-22
22a	0.95 <i>m</i>	34.4 <i>t</i>	CH <sub>2</sub>	H-20, H <sub>2</sub> -23, H-20b	C-20, C-23	Me-21
22b	1.65 <i>m</i>	—	—	H-20, H <sub>2</sub> -23	C-20	C-25, C-20, C-24
23a	0.92 <i>m</i>	26.1 <i>t</i>	CH <sub>2</sub>	H <sub>2</sub> -22, H-24	C-24	C-25, C-20
23b	0.92 <i>m</i>	—	—	H <sub>2</sub> -22, H-24	C-24	C-25, C-20
24	0.75 <i>m</i>	45.8 <i>d</i>	CH	H-25, H <sub>2</sub> -23, H <sub>2</sub> -28	C-25, C-28	C-29
25	1.45 <i>septet</i> (W <sub>1/2</sub> = 24.5)	29.2 <i>d</i>	CH	H-24, Me-26, Me-27	C-24, Me-26, Me-27	—
26	0.63 <i>d</i> (8.0)	20.4 <i>q</i>	Me	H-25	C-25	Me-27
27	0.62 <i>d</i> (8.0)	19.8 <i>q</i>	Me	H-25	C-25	Me-26
28a	1.02 <i>ddd</i> (14.0, 10.5, 8.0)	23.1 <i>t</i>	CH <sub>2</sub>	H-24, Me-29, H-24b	C-24, C-29	C-25
28b	1.06 <i>ddd</i> (14.0, 7.5, 10.5)	—	—	H-24, Me-29	C-24, C-29	C-25
29	0.65 <i>d</i> (10.5)	19.3 <i>q</i>	Me	H <sub>2</sub> -28	C-28	C-24
1'α	4.75 <i>d</i> (8.5)	104.7 <i>d</i>	CH	H-8'	C-8'	C-2', C-3, C-4'
2'	—	173.5 <i>s</i>	C	—	—	—
3'α	2.11 <i>dd</i> (6.5, 7.5)	50.2 <i>d</i>	CH	Me-10', H-4'	C-4', C-2'	C-5', C-8'
4'α	1.83 <i>dd</i> (6.5, 11.5, 9.5)	41.9 <i>d</i>	CH	H-3', H <sub>2</sub> -5'	C-3', C-8'	C-6', C-1'
5'a	1.09 <i>m</i>	33.9 <i>t</i>	CH <sub>2</sub>	H-5'b, H <sub>2</sub> -6'	C-4', C-6'	C-7'
5'b	0.83 <i>m</i>	—	—	H <sub>2</sub> -6', H-5'b	C-4', C-3'	C-7'
6'a	0.88 <i>ddd</i> (16.0, 8.5, 5.0)	24.3 <i>t</i>	CH <sub>2</sub>	H-6'b, H-7', H <sub>2</sub> -5'	C-5', C-7'	C-8', C-4'
6'b	1.41 <i>ddd</i> (16.0, 10.5, 7.5)	—	—	H-6'a, H-7'	C-5', C-7'	Me-9'
7'α	1.37 <i>dd</i> (7.5, 8.5)	36.1 <i>d</i>	CH	H <sub>2</sub> -6', Me-9', H-8'	Me-9', C-6', C-8'	—
8'α	1.27 <i>dd</i> (7.5, 9.5, 10.5)	39.5 <i>d</i>	CH	H-7', H-1'	C-1', C-7'	C-3, C-4'
9'β	0.86 <i>d</i> (7.5)	19.8 <i>q</i>	Me	H-7'	C-7'	C-8'
10'β	0.98 <i>d</i> (6.5)	20.4 <i>q</i>	Me	H-3'	C-3'	C-4'

\* Assignments were based on <sup>1</sup>H NMR, COSY, and HMQC experiments; coupling constants in Hertz are given in parentheses; *s*: singlet, *d*: doublet, *m*: multiplet, *t*: triplet, *brs*: broad singlet; \*\* DEPT chemical shifts are presented at  $\theta = 3\pi/4$  when methylene groups reaches negative maximum; C-multiplicities were established by DEPT experiment; \*\*\* The correlations in HMBC have been shown from protons to carbons.

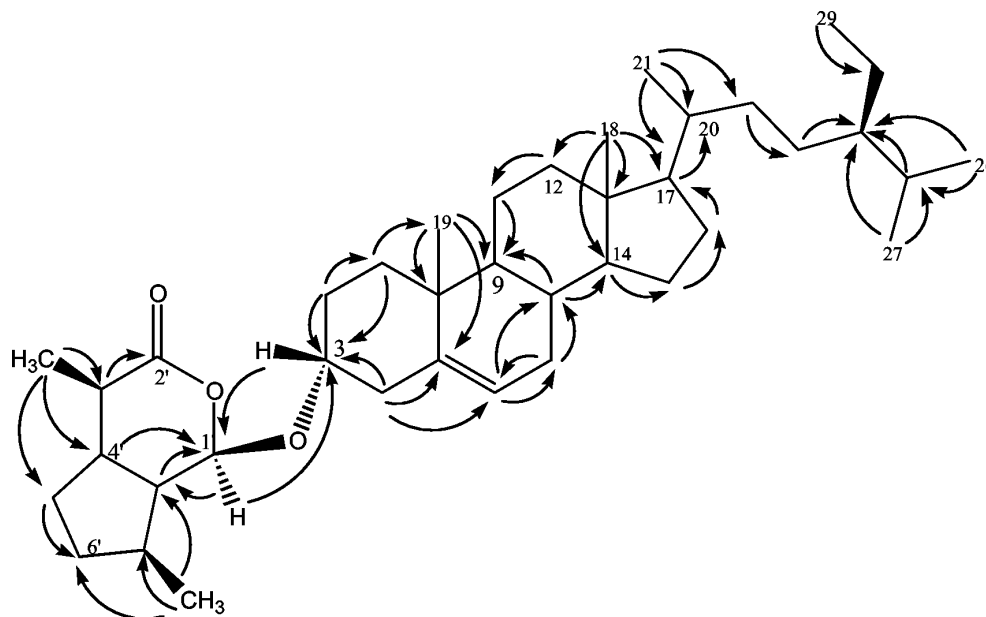
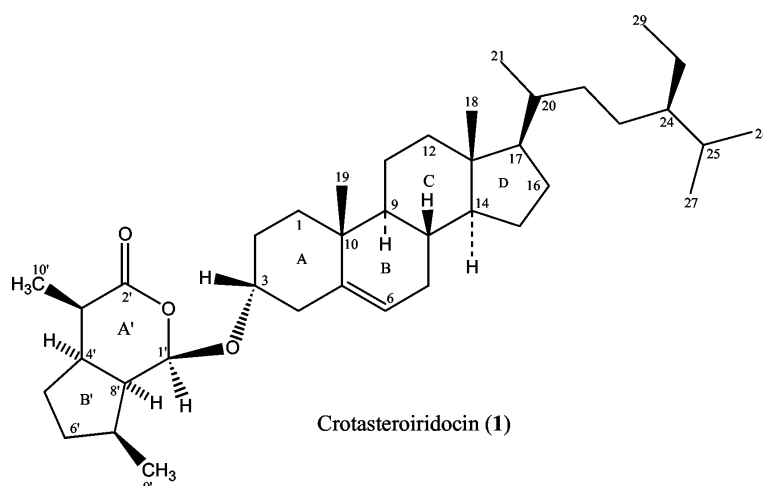


Fig. 1. Significant heteronuclear multiple bond correlations (HMBC) for crotasteroiridocin (**1**). Arrows point from proton to carbon.



mass spectrum displaying prominent peaks at  $m/z$  163, 177, and 161 (Scheme 1). The small coupling constants of the carbinolic proton indicated the  $\beta$ -orientation of the proton and  $\alpha$ -orientation of the hydroxyl oxygen atom [6]. Compound **1** did not give any acetylated derivative. The carbinolic proton exhibited correlations in  $^1\text{H}$ - $^1\text{H}$ -COSY spectrum with two methylene groups at  $\delta_{\text{H}} = 1.40, 1.78$  ( $\delta_{\text{C}} = 29.4$ ) assignable at position-2; and  $\delta_{\text{H}} = 2.02, 2.08$  ( $\delta_{\text{C}} = 38.6$ ) attributable to position-4. The  $\text{CH}_2$ -2, in turn, correlated with other methylene protons at  $\delta_{\text{H}} = 0.90, 1.65$  ( $\delta_{\text{C}} = 36.1$ )

that could be assigned to position-1. These positions were further substantiated by long-range couplings in HMBC spectrum, wherein H-3 correlated with C-2, C-4, C-5 ( $\delta_{\text{C}} = 140.3$ ) and C-1' ( $\delta_{\text{C}} = 104.7$ , another carbinolic carbon atom of iridoid unit);  $\text{CH}_2$ -2 correlated with C-1 and C-3;  $\text{CH}_2$ -1 with C-2, C-3, C-10 (36.7), and Me-19 (18.8) substantiating the structure of ring-A. The olefinic proton at  $\delta_{\text{H}} = 5.13$  ( $t$ ,  $J = 7.5$ ,  $\delta_{\text{C}} = 122.1$ ) assignable to position-6 showed correlations in  $^1\text{H}$ - $^1\text{H}$  COSY spectrum with a methylene group at  $\delta_{\text{H}} = 1.62, 1.05$  ( $\delta_{\text{C}} = 28.2$ ), which

was attributable to position 7. In turn, CH<sub>2</sub>-2 correlated with a methine proton at position 8 ( $\delta_{\text{H}} = 0.93$ ,  $\delta_{\text{C}} = 56.1$ ). H-8 displayed correlations with H-9 ( $\delta_{\text{H}} = 0.70$ ,  $\delta_{\text{C}} = 49.7$ ); and consequently H-9 with a methylene group at  $\delta_{\text{H}} = 1.25$ ,  $1.28$  ( $\delta_{\text{C}} = 21.1$ ) that could be assigned at position 11. The long-range couplings in HMBC spectrum further confirmed the above assignments (Table 1, Fig. 1). The methylene protons at position 11 showed correlations in the <sup>1</sup>H-<sup>1</sup>H-COSY spectrum with other methylene protons at  $\delta_{\text{H}} = 1.78$ ,  $1.81$  ( $\delta_{\text{C}} = 31.6$ ), which were attributable to position 12. The HMBC spectrum showed long-range correlations of H<sub>2</sub>-11 with C-9, C-12, C-8 and C-13, while H<sub>2</sub>-12 displayed with C-11, C-13 ( $\delta_{\text{C}} = 42.3$ ), C-14 ( $\delta_{\text{C}} = 56.7$ ) and Me-18 ( $\delta_{\text{C}} = 11.9$ ). In the same way, CH-14 showed correlations in the <sup>1</sup>H-<sup>1</sup>H-COSY spectrum with CH<sub>2</sub>-15 with ( $\delta_{\text{H}} = 1.78$ ,  $1.80$ ,  $\delta_{\text{C}} = 31.9$ ); CH<sub>2</sub>-15 with CH<sub>2</sub>-16 ( $\delta_{\text{H}} = 0.95$ ,  $1.80$ ,  $\delta_{\text{C}} = 39.7$ ); while CH<sub>2</sub>-16 showed correlations with CH-17 ( $\delta_{\text{H}} = 2.09$ ,  $\delta_{\text{C}} = 39.4$ ). The methine proton at position 17 displayed correlations in the COSY spectrum with another methine proton at  $\delta_{\text{H}} = 1.12$  ( $\delta_{\text{C}} = 31.9$ ) attributable to position 20, which in turn, showed correlation with a methyl group at  $\delta_{\text{H}} 0.71$  (d,  $J = 6.0$  Hz,  $\delta_{\text{C}} = 18.7$ ) that could be assigned at position 21. In this way, the presence of 5-ethyl-6-methyl-heptyl side chain assignable at C-17 was confirmed with the help of the <sup>1</sup>H-<sup>1</sup>H-COSY and HMBC spectra. The carbinolic proton at position-3 of the sterol moiety displayed long-range coupling in HMBC spectrum with another carbinolic proton at  $\delta_{\text{H}} = 4.75$  (d,  $J = 10.5$  Hz,  $\delta_{\text{C}} = 104.7$ ) assignable at position 1' of the iridoid unit. H-1' also showed long-range correlations with H-3, which indicated that the iridoid moiety was linked through an ether linkage ( $\nu_{\text{max}} 1025 \text{ cm}^{-1}$ ) with sterol unit *via* position 3. The high value of coupling constant ( $J = 10.5$  Hz) of the carbinolic proton indicated its  $\alpha$ -orientation, and consequently  $\beta$ -orientation of oxygen atom of the ether linkage. It also displayed correlation in <sup>1</sup>H-<sup>1</sup>H-COSY spectrum with a methine proton at  $\delta_{\text{H}} 1.27$  (ddd,  $J = 7.5$ ,  $9.5$ ,  $10.5$  Hz;  $\delta_{\text{C}} = 39.5$ ) attributable to position 8', which in turn, coupled with two methine protons at  $\delta_{\text{H}} = 1.83$  (ddd,  $J = 6.5$ ,  $9.5$ ,  $11.5$  Hz;  $\delta_{\text{C}} = 41.9$ ) assigned at position 4; and  $1.37$  (dd,  $J = 7.5$ ,  $8.5$  Hz;  $\delta_{\text{C}} = 36.1$ ) attributable to position 7', respectively. H-7', in turn, showed correlations with a methyl group at  $\delta_{\text{H}} = 0.86$  (d,  $J = 7.5$  Hz;  $\delta_{\text{C}} = 19.8$ ) placeable at position 9'; and with a methyl group at  $\delta_{\text{H}} = 0.88$ ,  $1.41$  ( $\delta_{\text{C}} = 24.3$ ) attributable to position 6'. Similarity H<sub>2</sub>-6' showed correlations with H<sub>2</sub>-5'

( $\delta_{\text{H}} = 1.09$ ,  $0.83$ ;  $\delta_{\text{C}} 33.9$ ); and H<sub>2</sub>-5', in turn, with H-4' ( $\delta_{\text{H}} = 1.83$ ,  $\delta_{\text{C}} = 41.1$ ). The above assignments of ring-B' were also substantiated further with the help of long-range couplings in HMBC spectrum. The methine proton at  $\delta_{\text{H}} 2.11$  (dd,  $J = 6.5$ ,  $7.5$  Hz;  $\delta_{\text{C}} = 50.2$ ) displayed <sup>1</sup>H-<sup>1</sup>H correlations with a methyl group  $\delta_{\text{H}} = 0.98$  (d,  $J = 6.5$  Hz,  $\delta_{\text{C}} = 20.4$ ) attributable to position-10'; and with a methine proton at position-4'. The long-range correlations in HMBC spectrum also supported the above sequence of ring-A', wherein H-3' showed correlations with C-4', C-2' ( $\delta_{\text{C}} = 173.5$ , C=O) and C-8'; Me-10' with C-3' and C-4' substantiating the above assignments. The high values of coupling constants of H-3' (dd,  $J = 6.5$ ,  $7.5$  Hz), H-4' (ddd,  $J = 6.5$ ,  $11.5$ ,  $9.5$  Hz), H-7' (dd,  $J = 6.5$ ,  $8.5$  Hz) and H-8' (ddd,  $J = 7.5$ ,  $10.5$ ,  $9.5$  Hz) indicated their  $\alpha$ -orientations, and hence  $\beta$ -orientation of both methyl groups, and *cis*-configuration of rings-A' and -B' [7]. The high down field shift of C-1' ( $\delta_{\text{C}} 104.7$ ) also supported that C-1' was linked with ether linkage with C-3, as well as with another oxygen atom of the lactone-ring-A'. The compound **1** was hydrolyzed with dil. HCl, which afforded two compounds namely, the sterol unit (**1a**) and iridoid moiety (**1b**). Their spectral data were close to the respective moieties of the original compound **1**.

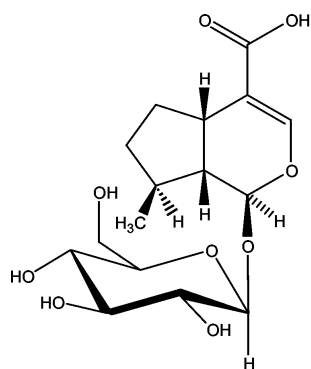
The structure of compound **1** was also supported by the fragmentation pattern of its mass spectrum, which displayed prominent peaks at  $m/z$  580 due to molecular ion, 413 and 396 (species *a*) due to sterol unit, 184 (species *b*) and 168 (species *d*) due to iridoid moiety, 114 (base peak, species *e*) due to a stable ion of ring-A' of the iridoid unit, 250 (species *f*) and 278 (species *g*) due to fragment obtained of rings A, B and rupture of ring-A'. Peaks at 193, (species *i*) and 195 (species *J*) due to rings A, B and rupture of ring-A'. Other peaks in the spectrum were also in good agreement with the proposed structure of the compound **1**.

Based on above chemical and spectral studies the sterol moiety (**1a**) was characterized as stigmast-5-ene-3- $\alpha$ -ol (3- $\alpha$ -isomer of  $\beta$ -sitosterol) [7a], whereas the iridoid unit (**1b**) was identified as 1- $\beta$ -hydroxy-isoiridomyrmecin [8, 9]. The compound **1** was thus characterized as 1'-hydroxy-isoiridomyrmecynyl-1'-O- $\beta$ -3-O- $\alpha$ -stigmast-5-ene and has been designated as crotasteroiridocin.

The compound **2** named as *bis*-desoxy-dihydromonotropein was acetylated to obtain as colorless needles (**2a**), which had a molecular formula C<sub>24</sub>H<sub>32</sub>O<sub>13</sub> (tetra acetate) as established on the basis of mass spectrum (528), <sup>13</sup>C NMR and DEPT spectra. Its IR

Table 2. *anti*-Inflammatory effects of crotasteroidocin (**1**) and tetra acetyl-bis-desoxy-dihydro-monotropein (**2a**) on right rat paw swelling induced by carrageenan.

Group <i>n</i> = 3	Dose mg/kg (i. p.)	Volume of paw (ml) after carrageenan administration				Total increase in paw volume (ml) after 3 h	Percent Inhibition
		0 h	1 h	2 h	3h		
Only Carrageenan	(1% w/v)	1.07 ± 0.05	1.68 ± 0.08	1.76 ± 0.06	1.88 ± 0.02	0.81 ± 0.07	—
Compound <b>1</b> + Carrageenan	10 mg	0.95 ± 0.09	1.50 ± 0.06	1.51 ± 0.06	1.56 ± 0.03 <i>t</i> = 2.277	0.60 ± 0.06	25.92%
Compound <b>2a</b> + Carrageenan	25 mg	1.07 ± 0.05	1.51 ± 0.04	1.63 ± 0.04	1.66 ± 0.07	0.58 ± 0.10	28.39%
Carrageenan + Phenylbutazone	100 mg	1.05 ± 0.11	1.22 ± 0.09	1.25 ± 0.06	1.32 ± 0.06 <i>t</i> = 6.277	0.27 ± 0.05*	66.66%

\*\* *P* < 0.01 student's *t*-test.Bis-desoxy-dihydro-monotropein (**2**)

spectrum indicated the presence of alcoholic ether linkage ( $1066\text{ cm}^{-1}$ ), carboxylic acid ( $3450\text{--}3250$ ,  $1665\text{ cm}^{-1}$ ) and double bond ( $1639$ ,  $771\text{ cm}^{-1}$ ). The  $^{13}\text{C}$  NMR and DEPT spectra [4] showed 24 carbon atoms for the molecule consisting of five methyls, three methylenes, nine methines, one olefinic methine, one carbinolic, one quaternary and one carboxyl carbon atom (in total  $\text{C}_{24}\text{H}_{32}$ ) for an aglycone moiety and one acetylated glucose unit. The sequential assignments of proton and carbon atoms were made with the help of  $^1\text{H}$ - $^1\text{H}$ -COSY and HMQC experiments [5] starting with the easily distinguishable olefinic and carbinolic protons. The spectral and chemical data of the compound were compared with the reported values, which were found similar with those of *bis*-desoxy-dihydro-monotropein **2** [7b, 10]. Hence the detail of the compound **2** does not need to present here, and thus only pertinent data have been given in the present paper.

#### Part B: *anti*-inflammatory activity

The results have been presented in Table 2. The crotasteroidocin (**1**) has shown a good *anti*-inflammatory activity, as it has decreased the edema by 25.9%

at a dose level of 10 mg/kg with respect to carrageenan (0.05 ml, 1%) used to cause inflammation in the paw of the rat, and 39.0% in comparison to standard phenylbutazone (66.6%) at the dose level of 100 mg/kg. Whereas bis-desoxy-dihydro-monotropein (**2**) has also shown a relatively good *anti*-inflammatory activity, by decreasing the edema 28.4% at a dose level of 25 mg/kg with respect to carrageenan, and 42.6% as compared to standard phenylbutazone (66.6%). The test compounds produced a significant inhibition of inflammation after 3 h. However, the dose dependent effect of the compounds could not be studied due to paucity of the compounds. Some other iridoid glucosides from the genus *Scrophularia* (*Scrophulariaceae*) have also been reported to possess *anti*-inflammatory activity [11], wherein the compounds containing cinnamoyl moiety at C-8 exhibited a potent activity in the range 20 to 30% at a dose of 10 mg/kg [12]. It may, therefore, be concluded that methyl group at position-8, as in the case of compound **2** (28.6%), may also play a significant role in displaying a good *anti*-inflammatory activity.

### Experimental Section

#### Part A: Chemistry

##### General

Melting points were determined on Mettler 9100 Electro thermal apparatus by open capillary method and are uncorrected. The IR spectra were recorded as KBr pellets on PYE UNICAM Spectrophotometer; mass spectra on a Finnegan MAT 300 mass spectrometer; 1D and 2D NMR on Bruker DRX 500 spectrometer in  $\text{CDCl}_3$  and  $\text{MeOH-}d_4$  using TMS as internal standard reference, chemical shift in  $\delta$  (ppm) and *J* values in Hz. The Centrifugal Preparative TLC was performed on a Chromatotron of Harrison Research Inc., USA using 4 mm rotor and silica gel PF-254 with  $\text{CaSO}_4 \cdot \frac{1}{2} \text{H}_2\text{O}$  as an adsorbent.

### Plant material

The aerial parts of *Crotalaria emarginella* Vatke were collected on April 22, 2004 from Agabat Tanha, Abha, Saudi Arabia, and identified by a taxonomist Dr. M. A. Rahman of the center, where a voucher specimen no. 14708 has been deposited in the herbarium for future reference.

### Extraction and isolation

Air-dried aerial parts (2.6 kg) were crushed to coarse powder and extracted exhaustively in a Soxhlet apparatus with 95% ethanol. The extracts were concentrated under reduced pressure to yield viscous masses (96.0 gm). The extract was extracted with petroleum ether, and the residue was dissolved in acetone. The acetone insoluble part (15.0 gm) was collected by filtration, and soluble part was treated with 2% tartaric acid, and consequently extracted with chloroform to remove the alkaloidal part. The aqueous layer was repeatedly extracted with *n*-butanol. The acetone insoluble part was dissolved in methanol and then treated with charcoal, and then filtered to remove the chlorophyll. The filtrate on drying was chromatographed on a column using hexane-ethyl acetate mixture as the eluent with increasing polarity. The eluent hexane-ethyl acetate (97:3) afforded the compound **1** (20 mg); while, the butanol fraction on column chromatography with chloroform-methanol (9:1) as the eluent yielded the compound **2** (140 mg).

**Crotasteroiridocin (1):** Obtained as colorless solid (20 mg), m. p. 170–71 °C. –  $R_f$  0.27 (EtOAc-hexane=1:9). –  $[\alpha]_D^{25} = -36$  ( $c = 0.04$ , MeOH). – IR (KBr):  $\nu_{\max} = 2947$  (CH<sub>3</sub>), 2872 (CH<sub>2</sub>), 1713 (C=O, lactone), 1670 (C=C), 1464, 1375, 1139 (C-O), 978 (C=C), 965, 802, 737 cm<sup>-1</sup>. – 1D and 2D NMR data (see Table 1). – MS: (70 eV):  $m/z$  (%) = 580 (10), 413 (7), 396 (23), 296 (15), 278 (71), 250 (31), 232 (2), 221 (10), 207 (3), 195 (9), 193 (8), 184 (12), 177 (14), 168 (10), 163 (10), 141 (15), 123 (5), 114 (100), 95 (20), 81 (25), 75 (9), 57 (20). – C<sub>39</sub>H<sub>64</sub>O<sub>3</sub> (580.4850). calcd. C 80.63, H 11.10; found C 80.64, H 11.11.

#### *Bis-desoxy-dihydro-monotropein (2):*

Obtained as viscous-solid (140 mg). It gave a positive Molisch's test for glycoside. It was acetylated with acetic anhydride and pyridine to get a colorless solid of tetra-acetate (**2a**, 150 mg).

**Tetra-acetyl-bis-desoxy-dihydro-monotropein (2a):** Obtained as colorless solid (150 mg). m. p. 170–71 °C. –  $R_f$  0.34 (CHCl<sub>3</sub>-MeOH =20:1).  $[\alpha]_D^{25} = 25$  ( $c = 0.06$ , MeOH). – IR (KBr):  $\nu_{\max} = 2960$  (CH<sub>3</sub>), 3450–3250 (broad, COOH), 2878 (CH<sub>2</sub>), 1748 (C=O, acetate), 1665 (C=O, carboxylic acid) 1639 (C=C), 1438, 1376, 1231 (C-O, ester), 1164, 1066 (broad, C-O, alcoholic), 909, 771 (C=C),

600 cm<sup>-1</sup>. – MS (EI, 70 eV):  $m/z$  (%) = 528 (5), 331 (50), 271 (20), 211 (15), 198 (10), 181 (20), 169 (100), 163 (30), 139 (25), 127 (40), 125 (20), 115 (14), 109 (90), 95 (20), 85 (8), 82 (25), 55 (20). – C<sub>24</sub>H<sub>32</sub>O<sub>13</sub> (528.0): calcd. C 54.54, H 6.10; found C 54.53, H 6.11.

### Part B: anti-inflammatory activity

The *anti-inflammatory* activity of the rare monoterpene glucoside, bis-desoxy-dihydro-monotropein (**2**) has not yet been reported so far. We have, therefore, screened the *anti-inflammatory* activity of its acetyl derivative (**2a**) together with irido-sterol, crotasteroiridocin (**1**) using rat paw method with reference to standard phenylbutazone, wherein they exhibited a good *anti-inflammatory* activity.

### Animals used

Wistar rats of either sex weighing 200–250 g were used for determining the activity. The animals were maintained at 23 ± 2 °C with 12 h light and dark cycle, fed Purina-rat chow diet supplied by Grain Silos and Flour Mills Organization, Riyadh, Saudi Arabia; and had free access of diet and water.

### Determinations of anti-inflammatory activity

Three rats each were allotted to different treatment groups. Edema was induced in the rats by injecting carrageenan (0.05 ml, 1% w/v in normal saline) into the sub-plantar tissue of the right hind paw using the reported method [13]. Paw volume was measured with a Plethysmometer (7140, Ugo Basile) before carrageenan injection and 0, 1, 2 and 3 h later. The edema was reported as the difference between the initial and final volume. The *anti-inflammatory* effect was expressed as the percentage inhibition compared with vehicle-treated animal with respect to a reference group treated with phenyl butazone (100 mg/kg). The test compounds with distilled water (0.1 ml/100 g rat) were administered orally 1 h before the injection of phlogistic agent.

### Statistical analysis

The results of the inflammatory activity are reported as mean ± S.E. Total variation, present in a set of data was estimated by one-way analysis of variance (ANOVA). Student's test and Dennett's test were used for determining the significance [14, 15].

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