

## X-Ray Diffraction Studies on Inhibitor of Platelet Aggregation Dictamnine

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**Summary:** In the course of our studies on the chemical constituents of the aerial parts of *Skimmia laureola*, a number of coumarins isogosperol (1) heraclenol (2), (+)-7-methoxy-6-(2'*R*-methoxy-3'-hydroxy-3'-methyl butyl) coumarin (3), 5, 8-dimethoxy coumarin-2*H*-1-benzopyran-2-one (4), 7-methoxy-6-[2'-oxo-3'-methyl butyl] coumarin (5), (+)-ulopterol (6), were isolated. In addition various quinoline alkaloids, 4-methoxy-1-methyl-3-(2'*S*-acetoxy-3'-ene butyl)-2-quinolone (7), 4-methoxy-1-methyl-3-(2'*S*-acetoxy-3'-hydroxy butyl)-2-quinolone (8), 3-hydroxy-2, 2, 6-trimethyl-3, 4, 5, 6-tetrahydro-2*H*-pyrano [3, 2-*c*] quinoline-5-one (9), 4-methoxy-1-methyl-3-(2'-oxo-3'-methyl butyl)-2-quinolone (10), 4-methoxy-1-methyl-3-(2'*S*-hydroxy-3'-ene butyl)-2-quinolone (11), methyl isoplatydesmine (12) ribalinin (13) and dictamnine (14) were also isolated from *Skimmia laureola*. The structures were established by spectroscopic studies. In this paper we report the X-ray diffraction studies on inhibitor of platelet aggregation dictamnine (14).

## Introduction

*Skimmia laureola* Hook (Rutaceae) is found in Kashmir and in the mountains of Northern Pakistan and it is used in indigenous system of medicine for the treatment of various ailments [1]. The soot obtained from the burning of leaves is inhaled for the treatment of body pain, fever and influenza by the local population of Northern Pakistan. The structures of the compounds (1-14) [2-12] were elucidated with help of extensive spectroscopic studies. The compound 14 was earlier isolated from *Dictamnus* plant [13-15]. This is the first report of its isolation from *Skimmia laureola*.

## Results and Discussion

Dictamnine (14), C<sub>12</sub>H<sub>9</sub>NO<sub>2</sub>, was isolated from a polar fraction by preliminary chromatography on a silica gel column. The UV spectrum displayed absorptions at 327, 314, 308, 237, 222 and 211 nm indicating the presence of a furoquinoline structure [13-15]. Spectroscopic analysis was not performed because this compound has been earlier isolated from *Dictamnus* plant [12-14]. A suitable crystal of 14 was selected isolated from *Skimmia laureola*, for the single-crystal X-ray diffraction studies [16]. A computer generated perspective drawing of 14 is shown in Fig. 1. All bond distances and angles are within expected values.

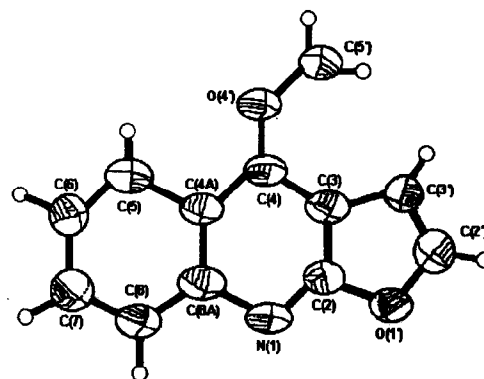
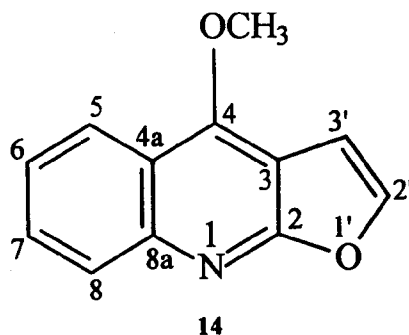


Fig. 1: A computer generated perspective drawing of the final X-ray model of dictamnine (14).

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Table-1: Atomic coordinates and equivalent isotropic displacement parameters for dictamnine (14).

	X	Y	Z	U (eq)
C (4A)	3310 (4)	8877 (2)	7354 (3)	44 (1)
C (4)	1452 (4)	9693 (2)	7722 (3)	44 (1)
C (5)	4355 (4)	8837 (2)	5776 (3)	51 (1)
O (1')	387 (4)	8836 (2)	11853 (2)	61 (1)
C (8A)	4127 (4)	8058 (2)	8597 (3)	46 (1)
N (1)	3194 (4)	8009 (2)	10146 (3)	52 (1)
C (8)	5975 (5)	7257 (2)	8206 (4)	57 (1)
C (3)	528 (4)	9658 (2)	9293 (3)	45 (1)
C (2)	1509 (4)	8781 (2)	10370 (3)	48 (1)
O (4')	796 (4)	10427 (1)	6479 (2)	58 (1)
C (6)	6117 (5)	8046 (2)	5446 (3)	59 (1)
C (3')	-1265 (5)	10259 (2)	10245 (3)	55 (1)
C (2')	-1267 (5)	9734 (2)	11718 (3)	61 (1)
C (7)	6941 (5)	7248 (2)	6680 (4)	61 (1)
C (5')	-1058 (6)	11274 (2)	6727 (4)	69 (1)

Table-2. Bond lengths for dictamnine(14).

C(4A)-C(4)	1.422(3)
C(4A)-C(8A)	1.429(3)
C(4)-O(4')	1.343(3)
C(4)-C(3)	1.386(3)
C(5)-C(6)	1.359(4)
O(1')-C(2)	1.370(3)
O(1')-C(2')	1.374(3)
C(8A)-N(1)	1.371(3)
C(8A)-C(8)	1.412(3)
N(1)-C(2)	1.295(3)
C(8)-C(7)	1.361(4)
C(3)-C(2)	1.422(3)
C(3)-C(3')	1.448(3)
O(4')-C(5')	1.424(3)
C(6)-C(7)	1.406(4)
C(3')-C(2')	1.335(4)

Table-3. Bond angles for dictamnine (14).

C (5)-C(4A)-C(4)	122.2 (2)	C(4)-C(3)-C(2)	115.7(2)
C(5)-C(4A)-C(8A)	118.5(2)	C(4)-C(3)-C(3')	139.2(2)
C(4)-C(4A)-C(8A)	119.2(2)	C(2)-C(3)-C(3')	105.1(2)
O(4')-C(4)-C(3)	127.0(2)	N(1)-C(2)-O(1')	120.1(2)
O(4')-C(4)-C(4A)	115.1(2)	N(1)-C(2)-C(3)	130.6(2)
C(3)-C(4)-C(4A)	117.9(2)	O(1')-C(2)-C(3)	109.3(2)
C(6)-C(5)-C(4A)	121.3(2)	C(2)-O(1')-C(2')	119.2(2)
C(2)-O(1')-C(2')	106.4 (2)	C(2)-O(1')-C(2)	120.0(2)
N(1)-C(8A)-C(8)	118.4(2)	C(2)-C(3)-C(3')	106.4(2)
N(1)-C(8A)-C(4A)	123.3(2)	C(3')-C(2')-O(1')	112.7(2)
C(8)-C(8A)-C(4A)	118.3(2)	C(8)-C(7)-C(6)	120.4(2)
C(2)-N(1)-C(8A)	113.3(2)		
C(7)-C(8)-C(8A)	121.4(2)		

## Experimental

### Plant Materials

The aerial parts of *Skimmia laureola*, Hook (20 Kg) was collected from Azad Kashmir in December 1992. A voucher specimen (KUH # 58106) was deposited in the Herbarium of Department of Botany, University of Karachi.

### General Experimental Procedures

The MS spectra were recorded on a Jeol HX-110 instrument. The  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectra were recorded in  $\text{CDCl}_3$  at 500 and 125 MHz, respectively, on a Bruker AM-500 NMR spectrometer. The UV and IR spectra were recorded on Shimadzu UV-240 and Jasco A-320 spectrophotometers, respectively. Optical rotations were measured on a polatron D polarimeter. The purity of the compounds was checked on TLC (si-gel, Merck PF<sub>254</sub>, 0.25 mm thickness). Melting points were determined in glass capillary tubes using Buchi 535 and Gallenkamp 30/MF-370 melting point apparatus.

### Extraction and Isolation

Air-dried aerial parts (20 kg dry weight) of *S. laureola* were extracted with EtOH (100 liters). The EtOH extract of the whole plant was concentrated to a gum (822 gm), dissolved in distd. water (aqueous extract) and extracted thoroughly with pet. ether (45 liters). The pet. ether soluble portion was evaporated under reduced pressure to yield a gum (66.92 gm). The remaining aqueous layer was acidified with acetic acid to pH 3 and then extracted with  $\text{CHCl}_3$ . The aqueous acidic layer was then made alkaline with  $\text{NH}_4\text{OH}$  to pH 12 and again extracted with  $\text{CHCl}_3$  (40 l). The  $\text{CHCl}_3$  soluble portion was dried over  $\text{Na}_2\text{SO}_4$ , filtered and evaporated to dryness in a vacuum to afford a crude alkaloidal mixture (74.96 gm). This mixture was chromatographed on a si-gel column (Merck, 70-230 mesh, 2015.01 gm) yielded an impure mixture containing compound 14.

### Isogospherol (1)

brown gum,  $[\alpha]_D^{29} = +40$  ( $c = 0.05$ ,  $\text{CHCl}_3$ ); UV  $\lambda_{\text{max}}$  (MeOH) 300, 248, 218 nm; IR ( $\text{CHCl}_3$ )  $\nu_{\text{max}}$  3395 (OH), 1716 (C=O), 1628 (C=C), 1590, 1580 (C=C conju.)  $\text{cm}^{-1}$ ;  $^1\text{H}$ - $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$ , reported in the literature. [2].

### Heraclenol (2)

Light brown gum;  $[\alpha]_D^{29} = +11^\circ$  ( $c = 2.0$ ,  $\text{CHCl}_3$ ); UV  $\lambda_{\text{max}}$  (MeOH) 300, 248 and 218 nm; IR ( $\text{CHCl}_3$ )  $\nu_{\text{max}}$  3400-3500 (br. OH), 1720 (C=O), 1625 (C=C)  $\text{cm}^{-1}$ ;  $^1\text{H}$ - $^{13}\text{C}$ -NMR;  $\delta$  reported in the literature [3].

(+)-7-methoxy-6-(2'R-methoxy-3'-hydroxy-3'-methyl butyl) coumarin (3).

White powdery mass;  $[\alpha]_D^{29} = 40^\circ$  ( $c = 0.05$ ,  $\text{CHCl}_3$ ); UV  $\lambda_{\text{max}}$  (MeOH) nm; IR ( $\text{CHCl}_3$ )  $\text{cm}^{-1}$ ;  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 500 MHz)  $\delta$ ;  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ , 125 MHz) [4].

5,8-Dimethoxy coumarin-2H-1-benzopyran-2-one (4).

white powdery compound;  $[\alpha]_D^{28} = 20^\circ$  ( $c = 0.05$ ,  $\text{CHCl}_3$ ); UV  $\lambda_{\text{max}}$  (MeOH) 207, 246, 274, 322 nm; IR ( $\text{CHCl}_3$ )  $\nu_{\text{max}}$  1719 (C = O), 169 (C = C), 1119 ( $\text{OCH}_3$ )  $\text{cm}^{-1}$ ;  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  reported in the literature [5].

7-Methoxy-6-[2'-oxo-3'-methyl butyl] coumarin (5).

Yellowish brown oily mass; UV  $\lambda_{\text{max}}$  (MeOH) 300, 223 nm; IR ( $\text{CHCl}_3$ ) 1720 (C = O), 1100 ( $\text{OCH}_3$ ), 1610 (C-H)  $\text{cm}^{-1}$ ; EIMS, HREI MS,  $^1\text{H-NMR}$ ;  $\delta$  reported in the literature [6].

(+)-Ulopterol (6).

Light brown gummy substance;  $[\alpha]_D^{29} = 10^\circ$  ( $c = 0.10$ ,  $\text{CHCl}_3$ ); UV (MeOH)  $\lambda_{\text{max}}$  nm ( $\log \epsilon$ ) 223, 327; IR ( $\text{CHCl}_3$ )  $\nu_{\text{max}}$   $\text{cm}^{-1}$  3400 (OH), 1720 (six-membered lactone carbonyl carbon), 1615 (C=C); EIMS, FDMS,  $^1\text{H-NMR}$ ;  $\delta$  reported in the literature [7,8].

4-Methoxy-1-methyl-3-(2'S-acetoxy-3'-ene butyl)-2-quinolone (7).

UV, IR, HREI MS:  $m/z$ ;  $^1\text{H-NMR}$ ,  $^{13}\text{C-NMR}$  [9].

4-Methoxy-1-methyl-3-(2'S-acetoxy-3'-hydroxy butyl)-2-quinolone (8).

UV, IR, HREI MS:  $m/z$ ;  $^1\text{H-NMR}$ ,  $^{13}\text{C-NMR}$  [9].

3-Hydroxy-2, 2, 6-trimethyl-3, 4, 5, 6-tetrahydro-2H-pyrano [3, 2-c] quinoline-5-one (9).

White amorphous substance,  $[\alpha]_D^{29} = -57$  ( $c = 0.138$ , MeOH); UV  $\lambda_{\text{max}}$  (MeOH) nm; IR ( $\text{CHCl}_3$ )  $\nu_{\text{max}}$ ; HREI MS:  $m/z$ ;  $^1\text{H-NMR}$  ( $\text{D}_2\text{O}$ , 400 MHz);  $^{13}\text{C-NMR}$  ( $\text{D}_2\text{O}$ , 75 MHz) [10].

4-Methoxy-1-methyl-3-(2'-oxo-3'-methyl butyl)-2-quinolone (10).

UV, IR, HREI, MS:  $m/z$ ;  $^1\text{H-NMR}$ ,  $^{13}\text{C-NMR}$  [6].

4-Methoxy-1-methyl-3-(2'S-hydroxy-3'-ene butyl)-2-quinolone (11).

UV, IR, HREI, MS:  $m/z$ ;  $^1\text{H-NMR}$ ,  $^{13}\text{C-NMR}$  [9].

Methyl isoplatydesmine (12).

White crystalline compound; m.p. = 73-75  $^\circ\text{C}$ ;  $[\alpha]_D = 40$  ( $c = 0.10$ ,  $\text{CHCl}_3$ ); UV (MeOH)  $\lambda_{\text{max}}$ ; IR: ( $\text{CHCl}_3$ )  $\nu_{\text{max}}$   $\text{cm}^{-1}$ ; EIMS:  $m/z$  (rel. int%); HREI MS: FDMS:  $m/z$ ;  $^1\text{H-NMR}$  ( $\text{D}_2\text{O}$ , 400 MHz);  $\delta$  reported in the literature [11].

Ribalinin (13)

The compound gave a red colour test with Dragendorff's reagent. Pale yellow gummy substance,  $[\alpha]_D^{29} = 10$  ( $\text{CHCl}_3$ ,  $c = 1$ ); UV: (MeOH)  $\lambda_{\text{max}}$ ; IR: ( $\text{CHCl}_3$ )  $\nu_{\text{max}}$   $\text{cm}^{-1}$ ; HREI MS:  $m/z$ ; FDMS:  $m/z$  259;  $^1\text{H-NMR}$ : ( $\text{CDCl}_3$ , 500 MHz);  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ , 125 MHz);  $\delta$  reported in the literature [12].

Dictamnine (14)

White crystalline compound;  $[\alpha]_D = 40$  ( $c = 0.10$ ,  $\text{CHCl}_3$ ), UV: IR: ( $\text{CHCl}_3$ ), EIMS: 199, FD MS:  $m/z$  199,  $^1\text{H-NMR}$  ( $\text{D}_2\text{O}$ , 400 MHz)  $\delta$ ; reported in the literature [13-15].

Single-Crystal X-ray diffraction Analysis of 14.

A well formed large crystal was used to collect the X-ray diffraction data. A perspective view of the molecule is shown in Fig. 1. A crystal of 14 having approximate dimension of 0.25x 0.30x0.25 mm was mounted on a glass fiber. All measurements were made on a Nicolet single-crystal X-ray diffractometer with graphite monochromated  $\text{CuK}\alpha$  radiations ( $\lambda = 1.54178 \text{ \AA}$ ). Cell constants and orientation matrix for data collection obtained from a least-squares refinement using the setting angles of 25 carefully centered reflections in the angles  $20.00 < 2\theta < 60.00^\circ$  corresponded to a primitive monoclinic cell with dimensions  $a = 4.800(0)$ ,  $b = 12.5800(10)$ ,  $c = 7.8770(10) \text{ \AA}$  and  $\beta = 99.95^\circ(10)$ ,  $V = 468.49(7) \text{ \AA}^3$  for  $Z = 2$  and formula weight = 199.20. The

calculated density was 1.412 Mg/ m<sup>3</sup>. The space group was found to be P2<sub>1</sub>. The data were collected at a temperature of 293 (2) K° using the  $\theta - 2\theta$  scan technique to a maximum  $2\theta$  value of 135°. Of the 1288 reflections which were collected, 934 were unique ( $R_{int} = 0.0667$ ). Equivalent reflections were merged. The intensities of three representative reflections were measured after every 97 reflections and no decay correction was applied. The linear absorption coefficient,  $\mu$ , for CuK $\alpha$  radiations was 0.796 mm<sup>-1</sup>. The data were corrected for Lorentz and polarization effects. Three standard reflections did not show any sign of decay during data collections. The structure was solved by direct methods SHELXTL [16]. The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included but not refined. The final cycle of full-matrix least-squares refinement was based on 934 observed reflections [ $1 > 2 \sigma(1)$ ] and 134 variable parameters were converged with unweighted and weighted agreement factor  $R = 0.046$  ( $R_w = 0.1099$ ). The asymmetric unit of 4 is composed of two independent molecules in the unit cell. There are no interactions between the molecules which are separated by normal Vander Waals distances. The bond distances and angles in the molecule as expected with mean values: C-O = 1.36 (1), C = C, 1.34 (1) and C-C (involving sp<sup>2</sup> hybridized C-atoms) = 1.41 (1) Å. Fractional coordinates of non-hydrogen atoms, bond lengths and bond angles of three independent molecules in an asymmetric unit are given in Tables-1, 2, 3, respectively.

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