Dictamnol, a New Trinor-Guaiane Type Sesquiterpene, from the Roots of *Dictamnus dasycarpus* TURCZ.

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A new trinor-guaiane type sesquiterpene, named dictamnol (1), and a steroid, pregnenolone (2), were isolated from *Dictamnus dasycarpus* TURCZ. On the basis of physicochemical evidence, the structure of dictamnol have been elucidated as 8α-methyl-2-methylene-1α,7α-bicyclo[3.5.0]dec-5-en-8β-ol.

Keywords *Dictamnus dasycarpus*; dictamnol; pregnenolone; trinor-guaiane type sesquiterpene

The roots of *Dictamnus dasycarpus* TURCZ. (Japanese name: Hakusen-pi; Rutaceae) are an active ingredient of Chinese medicines and have been used for the treatment of jaundice and various other diseases.

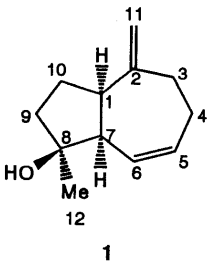
Although numerous chemical studies of the constituents of *Dictamnus* sp. have shown it to contain limonoids,[1-3] furanoquinoline alkaloids,[1,3,4] flavonoids,[5,6] coumarins,[6,7] and others compounds, we decided to isolate additional components in connection with its interesting pharmacological activities.

The crushed roots of *Dictamnus dasycarpus* TURCZ.[8] were extracted with MeOH and separated into neutral and carbonyl component fractions as shown in Chart 2. A compound **1**, named dictamnol, mp 72—73 °C, $[\alpha]_D$ +55° (c=0.1, MeOH), and a compound **2**, mp 192—194 °C, $[\alpha]_D$ +22° (c=0.1, $CHCl_3$), were isolated by repeated silica gel and alumina column chromatography from the neutral and carbonyl component fractions obtained.

Dictamnol (**1**) has the molecular formula $C_{12}H_{18}O$ (high resolution mass (HRMS) spectrum (m/z: 178.1337 (M^+)). The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **1** showed the presence of a tertiary methyl group on an oxygen-bearing carbon atom at δ 1.22 (3H, s), a proton of a hydroxy group at δ 1.25 (1H, s, D_2O-exchangeable) and four olefinic protons at δ 4.74 (1H, s), 4.82 (1H, s), 5.78 (1H, d, J=11.6 Hz), and 5.86 (1H, m), respectively. The ^{13}C-NMR spectrum of **1**, indicated the presence of four signals attributable to olefinic carbons at δ 107.25 (t), 129.92 (d), 131.80 (d), and 153.61 (s), but no carbonyl carbon signal was observed. These chemical shifts and the splitting patterns of the olefinic hydrogen and carbon signals in ^{1}H- and ^{13}C-NMR spectra indicated the presence of a 1,2-disubstituted double bond and an exomethylene group. The infrared (IR) spectrum showed a strong band at 3270 cm^{-1} assignable to the hydroxy group. Further, the hydroxy group was revealed to be tertiary by the presence of a quaternary carbinyl carbon signal at 80.37 (s) in ^{13}C-NMR. Dictamnol (**1**) is consequently a bicarbocyclic compound having a disubstituted double bond, an exomethylene group, and a quaternary carbon bearing a methyl and a hydroxy group.

The ^{1}H–^{13}C correlation spectroscopy (COSY) spectrum was then studied to identify the protons directly attached to the individual carbons. From the correlated peaks, we have been able to identify the pairs of carbons and directly bonded protons as shown in Table I. From the cross-peaks, by analysis of the network of ^{1}H–^{1}H COSY spectra due to vicinal couplings, the connectivity of the carbon atoms in **1** could be determined as shown in Fig. 1. However, at this stage, the connectivities of the quaternary carbons could not be determined (broken lines in Fig. 1).

In order to locate the quaternary carbons, the ^{1}H–^{1}H long range COSY spectrum with delay time was measured. The presence of the cross-peaks due to the long range couplings between the protons of C(11) and the protons of C(1) and C(3) show the connectivity of the quaternary carbon C(2) to the methine carbon C(1) and the methylene carbon C(3). In the same manner, the connectivity of the quaternary carbon C(8) to the methylene carbon C(9) and



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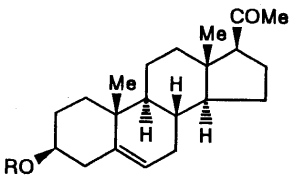

2: R= -H
3: R= -COMe

Chart 1

TABLE I. ^{1}H- and ^{13}C-NMR (500 MHz) Spectral Data of **1**[a]

Atom	δ_C	DEPT	δ_H	J_{H-H}
1	46.99	CH	2.43 m	
2	153.61	C		
3	36.64	CH_2	2.20 dd	13.6 ($J_{3\beta H-3\alpha H}$), 8.9 ($J_{3\beta H-4\beta H}$)
			2.56 t	13.6 ($J_{3\alpha H-3\beta H}$)
4	28.63	CH_2	2.11 m	
			2.28 m	
5	131.80	CH	5.86 m	
6	129.92	CH	5.78 d	11.6 (J_{6H-5H})
7	55.42	CH	2.38 d	11.6 ($J_{7\alpha H-1\alpha H}$)
8	80.37	C		
9	39.99	CH_2	1.73—1.78 m	
10	25.16	CH_2	1.89 m	
			1.80 m	
11	107.25	CH_2	4.74 s	
			4.82 s	
12	24.01	CH_3	1.22 s	
C(8)-OH			1.25 s	

a) All these assignments were confirmed by ^{1}H–^{1}H and ^{1}H–^{13}C COSY spectra.

crushed roots of *Dictamnus dasycarpus* TURCZ.
- extracted with MeOH and the resulting solution concentrated
- residue
  - absorbed on celite and extracted with ether
    - Celite
    - ether solution
      - dried and concentrated
      - residue
        - dissolved in ether and filtered
          - insoluble (limonoids)
          - soluble
            - washed with 5% HCl, 5% NaOH and water, then dried and concentrated
            - residue
              - treated with Girard P reagent
              - reaction mixture
                - poured into ice-water, adjusted to pH 7 with Na_2CO_3, and extracted with ether
                - aqueous layer (B)
                  - acidified with conc. HCl and extracted with ether
                  - aqueous layer
                  - ether layer
                    - dried and concentrated
                    - residue (carbonyl compounds)
                      - subjected to alumina chromatography
                      - pregnenolone (**2**)
                - ether layer (A)
                  - dried and concentrated
                  - residue (neutral compounds)
                    - subjected to silica gel chromatography
                    - fraxinellone
                    - dictamnol (**1**)
                    - sitosterol

Chart 2. Extraction and Separation

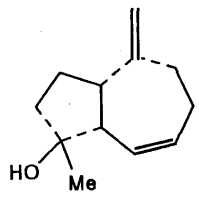

Fig. 1. Skeletal Structure of Dictamnol (**1**)

methine carbon C(7) was established. Thus, the skeletal structure of **1** was established as shown in Fig. 1.

The three dimensional (3D) structure of **1** was determined by a nuclear Overhauser and exchange spectroscopy (NOESY) spectrum, based on the skeletal structure as follows. The connectivity between isolated ^{1}H-coupled sectors was provided by essential stereochemical information as shown in Fig. 2. In particular, the presence of the cross-peaks between the protons of C(12) and the protons of C(1), C(6), and C(7), and the proton of C(1) and the proton of C(7) shows the close proximity of the protons of C(1), C(6), and C(7) to the protons of the methyl group, indicating that these protons protrude on one side of the molecule. Therefore, the relative 3D-structure of dictamnol is represented by formula **1** and this shows it to be a new trinor-guaiane type of sesquiterpene,[9] 8α-methyl-2-methylene-1α,7α-bicyclo[3.5.0]dec-5-en-8β-ol.

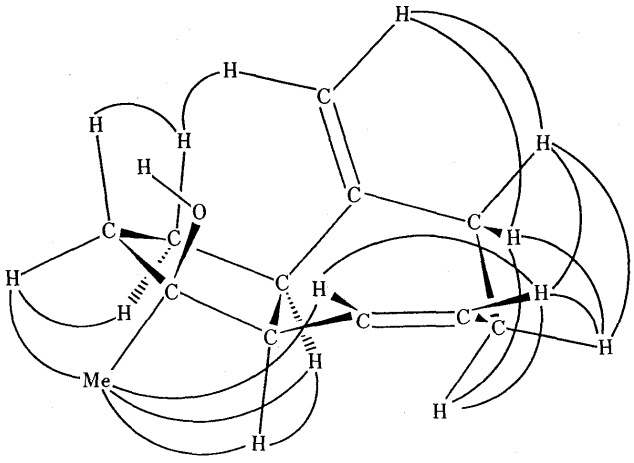

Fig. 2. NOESY Observed in Dictamnol (**1**)

Compound **2** has the molecular formula $C_{21}H_{32}O_2$ (HRMS *m/z*: 316.2411 (M^+)). The IR spectrum of **2** showed

absorption bands at 3506, 1684, and 1645 cm^{-1} due to a hydroxy and a carbonyl group and a double bond. The ^{1}H-NMR spectrum revealed the presence of two tertiary methyl groups at δ 0.64 (3H, s) and 1.01 (3H, s), a methyl group in an acetyl group at δ 2.12 (3H, s), a proton on an oxygen-bearing carbon at δ 3.51 (1H, br), and an olefinic proton at δ 5.36 (1H, d, J=4.9 Hz). Acetylation of **2** with acetic anhydride in pyridine gave the acetate **3**, mp 148.5—149.5 °C, $[\alpha]_D$ +13° (c=0.1, $CHCl_3$), $C_{23}H_{34}O_3$ (HRMS m/z: 298.2246 ($M^+ - CH_3CO_2H$)). Compound **2** is consequently a steroid with a hydroxy, an acetyl, and two methyl groups and a trisubstituted double bond. Compounds **2** and **3** were identified by direct comparison of their IR and ^{1}H-NMR spectra, optical rotations, and by mixed melting points with pregnenolone[10] and pregnenolone acetate[10] respectively. This is the first time that pregnenolone from *Dictamnus* sp. has been studied. This observation is an interesting isolation of a steroid from the plant kingdom.

Experimental

All melting points are uncorrected. Optical rotations were recorded with a JASCO DIP-140 polarimeter, IR spectra with a Hitachi 260-10 or JASCO FT/IR-8000 spectrometer, and ^{1}H- and ^{13}C-NMR spectra with a JEOL EX-90 or JEOL JNM-α500 spectrometer with tetramethylsilane as internal standard. ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY, ^{1}H–^{1}H long-range COSY, and NOESY spectra were obtained with the usual pulse sequence and data processing was performed with the standard JEOL software. HRMS spectra were recorded with a JEOL JMS-D 300 spectrometer. Elemental analyses were done by Kissei Pharmaceutical Company, Ltd., Matsumoto, Japan. Wakogel C-200 (silica gel) and Aluminium oxide 90 (alumina, Merck) and Merck Kieselgel G nach Stahl (silica gel) were used for column chromatography and thin-layer chromatography (TLC), respectively.

Isolation of Dictamnol (1) and Pregnenolone (2) from the Roots of *Dictamnus dasycarpus* Turcz. The crushed roots of *Dictamnus dasycarpus* Turcz.[8] (6.8 kg) were extracted three times with methanol and the resulting solution concentrated. The concentrated residue was absorbed on celite, then dried and extracted with ether. The ether solution was dried and concentrated. The residue was treated with ether again. The ether solution was washed with 5% HCl, 5% NaOH, and water, then dried and concentrated. A mixture of this residue (57.3 g), together with 1-(2-hydrazino-2-oxoethyl)pyridium chloride (Girard P reagent, 45 g) and acetic acid (110 g) in ethanol (1.1 l) were refluxed for 1 h. The reaction mixture was poured into ice-water and adjusted to pH 7 with Na_2CO_3. The solution was extracted with ether and separated into the ether layer (A) and the aqueous layer (B). A was washed with water, dried and concentrated and the residue subjected to silica gel chromatography using benzene followed by increasingly polar mixtures of benzene and chloroform. Rechromatography of fractions obtained from 50% benzene in chloroform afforded **1** (140 mg). B was acidified with concentrated HCl and then extracted with ether. The ether layer was washed with saturated Na_2CO_3 and water, then dried and concentrated. The residue was subjected to alumina chromatography using benzene followed by increasingly polar mixtures of benzene and chloroform. Rechromatography of fractions obtained from 50% benzene in chloroform afforded **2** (328 mg) (Chart 2).

Dictamnol (1) Colorless needles (sublimation), mp 72—73 °C. $[\alpha]_D$ +55° (c=0.1, MeOH). IR (Nujol) cm^{-1}: 3270, 1633. ^{1}H- and ^{13}C-NMR: see Table I. HRMS m/z: Calcd for $C_{12}H_{18}O$ (M^+): 178.1355. Found: 178.1337. *Anal.* Calcd for $C_{12}H_{18}O$: C, 80.54; H, 10.18. Found: C, 80.54; H, 9.82.

Pregnenolone (2) Colorless needles (ether), mp 192—194 °C. $[\alpha]_D$ +22° (c=0.1, $CHCl_3$). IR (KBr) cm^{-1}: 3506, 1684, 1645. ^{1}H-NMR ($CDCl_3$) δ: 0.64 (3H, s, -Me), 1.01 (3H, s, -Me), 2.12 (3H, s, –COMe), 0.80—2.64 (20H, m, methine and methylene H), 3.51 (1H, br, >CHOH), 5.36 (1H, d, J=4.9 Hz, olefinic H). HRMS m/z: Calcd for $C_{21}H_{32}O_2$ (M^+): 316.2401. Found: 316.2411. *Anal.* Calcd for $C_{21}H_{32}O_2$: C, 79.70; H, 10.19. Found: C, 79.52; H, 9.99. This compound **2** was identified by comparing the IR and ^{1}H-NMR spectra and optical rotations, and by mixed melting points with pregnenolone.[10]

Pregnenolone Acetate (3) Acetic anhydride (3 ml) was added to a solution of **2** (50 mg) in pyridine (1 ml) and the resulting solution was allowed to stand overnight at room temperature. The reaction mixture was poured into ice-water and extracted with ether. The ether layer was washed with saturated $NaHCO_3$, 10% HCl, and water, then dried and concentrated. The residue was subjected to silica gel chromatography. The eluate with 20% hexane in ethyl acetate gave 45 mg (79.4%) of **3** as colorless needles (hexane–ether), mp 148.5—149.5 °C. $[\alpha]_D$ +13° (c=0.1, $CHCl_3$). IR (KBr) cm^{-1}: 1728, 1705, 1674. ^{1}H-NMR ($CDCl_3$) δ: 0.64 (3H, s, -Me), 1.02 (3H, s, -Me), 2.04 (3H, s, –OCOMe), 2.12 (3H, s, –COMe), 1.02—2.70 (20H, m, methine, methylene H), 4.65 (1H, br, >CHOCOMe), 5.38 (1H, d, J=3.7 Hz, olefinic H). HRMS m/z: Calcd for $C_{21}H_{30}O$ ($M^+ - CH_3CO_2H$): 298.2276. Found 298.2246. This compound **3** was identified by comparing the IR and ^{1}H-NMR spectra and optical rotations, and by mixed melting points with pregnenolone acetate.[10]

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