A New Active Compound from *Centaurea* Species

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The extract containing sesquiterpene lactones of *Centaurea iberica* (Asteraceae) isolated was separated and a steroidal compound, which is stigmast-1,5-dien-3β-ol, was purified. The chemical structure was established based on spectroscopic data (UV, IR, MS, ^{1}H NMR, ^{13}C NMR). Both the extract and the compound showed significant antioxidant and antimicrobial activities.

Key words: Centaurea, Antimicrobial, Stigmast-1,5-dien-3β-ol

Introduction

Centaurea (Asteraceae) species are known for their potential active compounds. The Asteraceae family contains many medicinal species and isolated biological active compounds. *Centaurea* is the most favorite genus containing active natural compounds, such as sesquiterpene lactones, flavonoids, phenolic acids and steroidal structures (Santos *et al.*, 1995; Sarker *et al.*, 1998; Öksüz and Serin, 1997). *Centaurea iberica* Trev. ex Sprengel is known as "timur dikeni" and is used as antidiabetic, appetizer, mucolitic, antipyretic, stomachic, antispazmodic against mensturation pains in traditional Turkish folk medicine (Baytop, 1999). Previous investigations on different extracts of this plant have shown cytotoxic and antibacterial activities. A cytotoxic activity has been reported for the sesquiterpene lactone extract of *C. iberica* with the brine shrimp (*Artemia salina*) method (Solis *et al.*, 1993). Further investigations of the extract showed that the purified compound is more antioxidant than its extract against authentic samples which were butylhydroxyanisol, α-tocopherol and ascorbic acid.

Material and Methods

Plant material

Centaurea iberica Trev. ex Sprengel was collected from Çatalca, Turkey in June 1994. A voucher specimen is deposited at the Faculty of Pharmacy Herbarium, Marmara University, Turkey (MARE 4473). The plant was identifed by Prof. Dr. Ertan Tuzlacı.

Isolation and identification

The air-dried and powdered plant (1.1 kg) was percolated with petroleum ether/diethyl ether/EtOH (1:1:1) (v/v/v) for 3 d in room temperature. The filtered extract was concentrated under reduced pressure (15 g). The final extract was chromatographed on a silica gel and a Sephadex LH-20 column, respectively. The compound (Fig. 1) was obtained from the 2nd fraction where petrolum ether/CH_2Cl_2 (90:10 v/v) was used as the elution solvent. The fraction was monitored by TLC on silica gel (Merck) plates using petroleum ether/toluene (4:1) as the mobile phase.

Stigmast-1,5-dien-3β-ol ($C_{29}H_{48}O$): Amorphous powder. – UV ($CHCl_3$): λ_{max} = 248 nm. – IR (KBr): ν_{max} = 3450 (OH), 2957 (C-C), 1640 (C=C), 1463, 1380, 1295 (C-O), 1080, 1035, 795 cm^{-1}. – EIMS: *m/z* = 412 $[M]^+$ ($C_{29}H_{48}O$) (100), 397 (23.2), 394 (33.9), 379 (18.2), 327 (25.5), 303 (27.9), 248 (26.7), 226 (14.5), 197 (8.9), 164 (17.3), 144 (27.0), 94 (27.3), 83 (26.2), 81 (33.5), 70 (23.7), 69 (30.0), 55 (70.5). – ^{1}H NMR (500 MHz): δ = 5.35 (1H, d, *J* = 5.1 Hz, H-6), 5.14 (1H, d, *J* = 8.4 Hz, H-1), 5.02 (1H, dd, *J* = 8.4, 8.4 Hz, H-2), 3.52 (1H, m, H-3), 1.00 (3H, brs, Me-19), 0.97 (3H, d, *J* = 6.6 Hz, Me-21), 0.88 (3H, d, *J* = 6.5 Hz, Me-29), 0.84 (1H, d, *J* = 6.0 Hz, Me-26), 0.82 (3H, d, *J* = 6.0 Hz, Me-27), 0.68 (3H, brs, Me-18). – ^{13}C NMR (400 MHz): δ = 138.41 (C-1), 129.3 (C-2), 71.84 (C-3), 42.35 (C-4), 140.8 (C-5), 121.8 (C-6), 29.76 (C-7), 31.71 (C-8), 50.21 (C-9), 36.21 (C-10), 21.15 (C-11), 37.33 (C-12), 39.85 (C-13), 56.93 (C-14), 24.34 (C-15), 28.31 (C-16), 56.13 (C-17), 11.92 (C-18), 19.45 (C-19), 34.0 (C-20), 18.85 (C-21),

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31.97 (C-22), 26.16 (C-23), 45.9 (C-24), 29.23 (C-25), 19.11 (C-26), 19.88 (C-27), 23.18 (C-28), 12.04 (C-29).

Bioactivity test methods

DPPH method

500 mg/l samples were mixed with 1 ml of a 1 mM methanolic solution of DPPH radical. The mixtures were shaken and left to stand for 30 min in dark at room temperature. The absorbance of the final solutions was measured at 517 nm against a blank sample. Ascorbic acid was used as control (Blois, 1958).

Reducing power method

500 mg/l samples were mixed with 2.5 ml of phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide, and the mixtures were incubated at 50 °C for 30 min. After 2.5 ml of 10% trichloroacetic acid (w/v) were added, the mixtures were centrifuged at 3000 rpm for 10 min. The upper layer (5 ml) was mixed with 5 ml of deionized water and 1 ml of 0.1% ferric chloride, and the absorbance of the mixture was measured at 700 nm against a blank sample. Butylated hydroxyanisol and α-tocopherol were used as controls (Özgen *et al.*, 2003).

These methods were used for testing the antioxidant activity of the extract and the compound. Agar well diffusion and tube dilution methods (National Committee, 1993) were used to determine antimicrobial activities. Meropenem (antibacterial) and fluconazole (antifungal) were used as standard drugs.

Results

C. iberica was investigated for the first time phytochemically. A steroidal compound, that had been isolated from *Desmotrichum fimbriatum* (Orchidaceae) for the first time (Ali *et al.*, 2003) and named as desmosterol, was found by us in *Centaurea iberica*, although it had not been reported previously in this genus. The extract has more antioxidant capacity than the compound. The antioxidative quantitative data are 80% for the compound, 84% for the extract, 98% for ascorbic acid with the DPPH method and 0.76 for the compound, 0.80 for the extract, 1.0 for butylhydroxyanisol, 0.98 for α-tocopherol with the reducing power method. The cytotoxicity of the extract has been controlled by the brine shrimp (*Artemia salina*) method. It has been found to be active (Ulusoylu *et al.*, 2001). Both the compound and the extract were investigated microbiologically. The compound exerted more activity than the extract, especially on *S. epidermidis* (MIC: 3.4 μg/ml), *S. aureus* (MIC: 6.8 μg/ml), *P. aeruginosa* (MIC: 6.8 μg/ml) against meropenem (MIC: 1.56, 3.125, 0.25 μg/ml, respectively). It certainly is a novel compound from the *Centaurea* genus and the Asteraceae family.

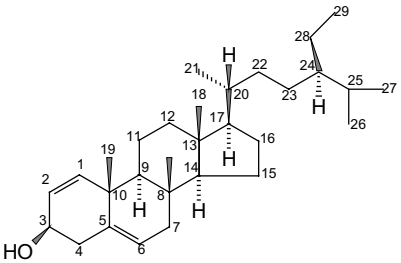

Fig. 1. Structure of stigmast-1,5-dien-3β-ol.

Discussion

The compound (Fig. 1) was isolated from the petroleum ether/ethanol (90:10) fraction. It gave a positive result in the Liberman-Buchard test for sterols. 3450 cm^{-1} (hydroxy group), 1640 cm^{-1} (unsaturation), 1380, 1308, 1080 cm^{-1} (dimethylisopropyl group) were presented by IR spectra. The EIMS spectrum exhibited diagnostically important fragment ions at *m/z* 226, 197 due to C_8/C_{14} and C_{11}/C_{12} fission and the ions at *m/z* 248 and 164 arose due to C_8/C_{14} and C_9/C_{11} fission. The fragments suggested that the compound was a C_{29} sterol. Possessing one hydroxy group at ring A/B which was placed at C-3 on the basis of the biosynthesis pathway, a C-10 saturated side-chain and a double unsaturated steroidal structure is concluded one of it was at C-2. The presence of important peaks at *m/z* 69 and 83 indicated the existence of one double bond at C-5. That result confirmed the location of a hydroxy group at C-3. All data obtained were controlled by literature data (Ali *et al.*, 2003).

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