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A NEW COMPONENT FROM *Eupatorium cannabinum*

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A new compound of formula $C_{28}H_{48}O$ with mp 179-180°C (aqueous ethanol) that was called eucanbin was isolated pure by column chromatography of the ethanol extract of the aerial part of Eupatorium cannabinum *L. The structure 24α-methylcholest-20(21)-en-3β-ol was assigned based on chemical and spectral data.*

Key words: *Eupatorium cannabinum*, Asteraceae, 24α-methycholest-20(21)-en-3β-ol.

Aerial parts, rhizomes, and roots of *Eupatorium cannabinum* L. (Asteraceae) are used in traditional and experimental medicine and homeopathy. According to the literature, essential oil, sesquiterpenoids, saponins, alkaloids, phenolcarboxylic acids, coumarins, flavonoids, triterpenoids, carbohydrates, steroids, and others have been isolated from various parts of *E. cannabinum* [1-12]. This species indigenous to Azerbaidzhan has not been studied in a chemical sense. Therefore, we studied the aerial part of *E. cannabinum* collected in the village Ilisu of Gakh Region, Republic of Azerbaidzhan, in July 2007 during mass flowering.

Total extracted compounds obtained by ethanol extraction of *E. cannabinum* were separated by chromatography over a column of Al_2O_3 to isolate a pure crystalline compound **1**, R_f 0.67, $C_{28}H_{48}O$, mp 179-180°C.

The IR spectrum of **1** exhibited absorption bands of OH groups (3350 cm^{-1}) and a double bond (1645) at the characteristic frequencies. The double bond of the compound was according to the IR spectrum (880 cm^{-1}) apparently an *exo*-methylene.

Acetylation of **1** gave compound **2** of formula $C_{30}H_{50}O_2$, mp 210-212°C. Its IR spectrum contained bands characteristic of an acetyl (1730, 1250 cm^{-1}) and a double bond (1645, 890).

The PMR spectrum of **1** in the methyl region (0.8-1.3 ppm) exhibited resonances belonging to five methyls, including two from isopropyl, one secondary, and two angular methyls. A 1H triplet at 3.5 ppm was characteristic of a *gem*-hydroxyl proton. Singlets at 4.8 (1H) and 4.85 (1H) belonged to CH_2=C< protons. Other resonances were not observed at weak field in the PMR spectrum. The PMR spectrum of **2** showed that the resonance of the *gem*-hydroxyl proton that was observed in the spectrum of **1** (at 3.5 ppm, 1H, >CH–OH) had undergone a paramagnetic shift and appeared at 4.7 ppm (q, J_1 = 5.06, J_2 = 11 Hz). The resonance of the acetyl in the spectrum of **2** was observed as a 3H singlet at 2.1 ppm. The spectral data indicated that **1** contained one secondary hydroxyl and one methylene double bond.

PMR data for characteristic groups of steroid **1** isolated from *E. cannabinum* are given below:

*C atom**	δ_H, *J/Hz*	*C atom**	δ_H, *J/Hz*
3	3.50 (1H, q, J_1 = 5.06; J_2 = 11)	24	1.10 (3H, d, J = 6.5)
18	1.01 (3H, s)	26	0.90 (3H, d, J = 6.7)
19	1.08 (3H, s)	27	0.902 (3H, d, J = 7.2)
21	4.80 (1H, s); 4.85 (1H, s)		

*Assignments of methyl resonances may be interchanged.

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Thus, a comparison of the physicochemical and spectral (IR, PMR, ^{13}C DEPT 135) properties of the studied compound with the published data [13-18] led to the conclusion that it was new. The name eucanbin (**1**) was proposed for it.

The ^{13}C NMR spectrum of the compound was recorded with full suppression of spin—spin coupling to protons in order to determine the number of C atoms in the molecule. The spectrum showed 28 singlets belonging to 28 C atoms (10-160 ppm). This agreed with the number of C atoms in the elemental composition of the compound.

Resonances of all protonated (except non-protonated C_{10}, C_{13}, and C_{20}) C atoms appeared in the ^{13}C DEPT 135 spectrum. Thus, the molecule had resonances for five C atoms of methyls at 16.0, 17.2, 17.5, 18.0, and 20.1 ppm according to the DEPT 135 spectrum. Resonances at 19.8, 22.8, 27.0, 27.5, 28.0, 29.2, 31.0, 35.5, 39.5, 40.0, 40.1, and 108.5 ppm that belonged to methylene C atoms indicated that the structure included 12 methylenes. Of them, the last (108.5 ppm) corresponded to a C atom of a double-bond methylene (CH_2=C<). Resonances of methine C atoms were observed in the spectrum at 26.0-80.0 ppm (26.5, 29.5, 40.2, 40.5, 49.5, 51.5, 57.0, and 79.0 ppm). The resonance at 79.0 ppm belonged to a single oxygenated C atom (>CH–OH) and indicated that the structure contained a secondary hydroxyl.

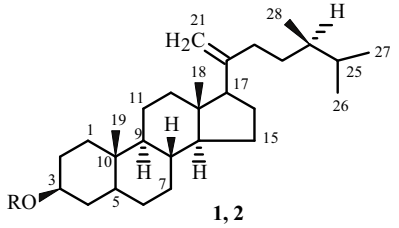
The ^{13}C NMR spectrum of characteristic groups of **1** is given below:

C atom	δ_C	*C atom*	δ_C
3	79.0	19	17.2
20	154.0	26	17.5
21	108.0	27	20.1
18	16.0	28	18.0

It can be assumed by analyzing the results that the studied compound was based on the C skeleton of saturated campesterol (5,6-dihydrocampestrol) [19]. A comparison of the chemical shifts in both the PMR and ^{13}C NMR spectra of structurally similar compounds, i.e., campesterol, brassicasterol, 22-dehydrocampesterol, and other steroidal compounds [13-18], revealed substantial differences that were explained by the different conditions under which the spectra were recorded. NMR spectra of eucanbin were recorded in deuterated pyridine and caused a paramagnetic shift of the resonances in its NMR spectra [20].

The double bond (methylene) could be placed in one of the positions C_{20}–C_{21}, C_{24}–C_{25}, C_{26}–C_{27}, and C_{26}–C_{28}. If the double bond were situated in either of the last two positions, a 3H singlet for a vinyl methyl (CH_3–C=CH_2) would have appeared at ~2.0 ppm. Furthermore, the resonance of the C24 methyl would usually undergo a paramagnetic shift due to the influence of the double bond located in the allylic position. Resonances of the isopropyl would undergo a paramagnetic shift if the double bond were at C_{24}–C_{28}. Therefore, the most probable position for the double bond was C_{20}–C_{21}.

Regarding the stereochemistry of the C_{24} methyl of eucanbin, it should be noted that it probably has the α-orientation. This is due to the fact that the C_{24} methyl in campesterol, which was also isolated from *E. cannabinum* [19], has the α-orientation. Therefore, campesterol and eucanbin, being products of the same biosynthetic process occurring in *E. cannabinum*, have the same stereochemistry at C_{24} from the viewpoint of biogenesis. Therefore, eucanbin was most probably the 5,6-dihydro-20(21)-dehydro derivative of campesterol and had the structure 24α-methylcholest-20(21)-en-3β-ol (**1**).



1, 2

1: R = H
2: R = OCCH$_3$

EXPERIMENTAL

General Comments. All solvents were freshly distilled. The purity of compounds was established by chromatography on Silufol UV-254 plates using $CHCl_3$. IR spectra in mineral oil were recorded on a UR-20 spectrophotometer; PMR and

^{13}C NMR spectra in deuterated Py, on a Bruker spectrometer (300 MHz for ^{1}H; 75, ^{13}C). Chemical shifts are given on the δ-scale with TMS internal standard.

Melting points were determined on a Boetius stage.

Isolation of Eucanbin. Extraction of Plant Material. Ground air-dried aerial parts of *E. cannabinum* (0.450 g) were extracted twice with ethanol (96%) for 3 d each. The alcohol was filtered off and distilled in a rotary evaporator using a water bath to afford total extracted compounds (33 g, 7.33% yield). Part (15.0 g) of the extracted compounds was chromatographed over a column of Al_2O_3 (h = 90 cm, d = 3.5 cm, Al_2O_3 neutral, Brockmann activity III-IV). The volume of each fraction was 100 mL. The column was eluted with hexane (29 fractions), hexane:benzene (4:1, 19 fr.; 3:1, 3 fr.; 3:2, 13 fr.; 1:1, 3 fr.), benzene (18 fr.), benzene:$CHCl_3$ (3:2, 4 fr.; 2:3, 5 fr.), $CHCl_3$ (37 fr.), and $CHCl_3$:C_2H_5OH (95:5, 9 fr.; 80:20, 3 fr.). Fraction 20, which was eluted by hexane, afforded a pure crystalline compound, $C_{28}H_{48}O$, mp 179-180°C (aqueous ethanol), λ_{max} 3350, 1645, 880 cm^{-1}.

Acetylation. The compound (0.1 g) was dissolved in Py (5 mL), treated with acetic anhydride (6 mL), heated on a water bath for 5 h, diluted with water (5 mL), and evaporated in a ceramic dish on a water bath. The solid was dissolved in $CHCl_3$ (5 mL), filtered through a layer of Al_2O_3 (10 cm, activity III-IV), and washed with $CHCl_3$. The resulting crystalline acetyl derivative was recrystallized from aqueous ethanol, $C_{30}H_{50}O_2$, mp 210-212°C, ν_{max} 1730, 1250, 890 cm^{-1}.

REFERENCES

1. V. I. Chopik, L. G. Dudchenko, and A. N. Krasnova, *Wild Useful Plants of Ukraine* [in Russian], Kiev, 1983.
2. A. Judzentiene, *J. Essent. Oil Res.*, **19**, 5, 403 (2007).
3. R. Hegnauer, *Chemotaxonomie der Pflanzen*, Basel, Stuttgart (1966), **3**, **4**, 743, 5511.
4. B. Drozdz, H. Grabarczyk, Z. Samek, M. Holub, M. Herout, and F. Sorm, *Collect. Czech. Chem. Commun.*, **37**, 5, 1546 (1972).
5. C. Zdero and F. Bohlmann, *Planta Med.*, **53**, 2, 169 (1987).
6. T. A. Pkheidze and O. S. Madaeva, *Study of Certain Saponin-Bearing Plants Growing in Georgia, Biologically Active Compounds of the Flora of Georgia* [in Russian], No. 10, 1965, pp. 223-270.
7. L. Pomili, *Riv. Ital. Essenze, Profumi, Piante Off., Aromi, Saponi, Cosmet., Aerosol (Ed. Eur.)*, **58**, 4, 189 (1976).
8. I. M. Kikvidze, T. A. Khverelidze, and K. S. Mudzhiri, *Alkaloid-Bearing Plants of Certain Regions of Eastern Georgia, Biologically Active Compounds of the Flora of Georgia* [in Russian], No. 12, 1973, pp. 94-96.
9. P. S. Chikov, *Medicinal Plants* [in Russian], Moscow, 1989.
10. M. Oswiecimska and J. Sendra, *Diss. Pharm. Pharmacol.*, **24**, 5, 475 (1972).
11. T. G. Sagareishvili, M. D. Alaniya, and E. P. Kemertelidze, *Khim. Prir. Soedin.*, 106 (1981).
12. Kh. Kh. Khalmatov, *Wild Medicinal Plants of Uzbekistan* [in Russian], Tashkent, 1964.
13. T. W. Goodwin and E. I. Mercer, *An Introduction to Plant Biochemistry*, 2nd Ed., Pergamon, Oxford, UK, 1982.
14. G. Cabrera, J. A. Palermo, A. M. Selds, E. G. Gros, and J. C. Oberti, *Phytochemistry*, **30**, No. 4, 1239 (1991).
15. J. L. C. Wright, *Phytochemistry*, **20**, No. 10, 2403 (1981).
16. M. Tandon, Y. N. Shukla, and R. S. Thakur, *Phytochemistry*, **29**, No. 5, 2957 (1990).
17. T. Matsumoto, S. T. Shigemoto, T. Iton, T. Iida, and A. Nishioka, *Phytochemistry*, **22**, No. 3, 789 (1983).
18. T. Akihisa, N. Shimizu, P. Ghosh, S. Thakur, F. U. Rosenstein, T. Tamura, and T. Matsumoto, *Phytochemistry*, **26**, No. 6, 1693 (1987).
19. G. C. Levy and G. L. Nelson, *Carbon-13 Nuclear Magnetic Resonance for Organic Chemists*, Wiley-Interscience, New York, 1972.
20. R. Aquino, M. D'Agostino, F. De Simone, and O. Schettino, *Boll. Soc. Ital. Biol. Sper.*, **61**, 8, 1087 (1985).