The known iridoids and phenylpropanoid compounds were identified by comparison with authentic samples (pmr and ir spectra superimposable, α_D identical).

Full details of the isolation and identification of the compounds are available on request to the authors.

ACKNOWLEDGMENTS

The authors are grateful to Prof. O. Sticher for an authentic sample of eukovoside; we are also grateful to Mr. S. Ruggieri for the help in the extraction of the plant material.

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Received 21 July 1983

6-METHOXYLATED AND *C*-GLYCOSYL FLAVONOIDS FROM *CENTAUREA* SPECIES

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In a previous study, we described the sesquiterpene lactones from *Centaurea behen* (1), *Centaurea kotschyi* (2), and the cytotoxic flavones from *Centaurea urvillei* (3).

As a part of our continuing investigation, we now report the isolation, identification, and antibacterial activity of the flavonoids obtained from *Centaurea virgata* Lam., *Centaurea kilea* Boiss., and *Centaurea inermis* Velen.

A total of ten flavonoids were obtained from the samples of the above-mentioned species. Apigenin, the major compound, and jaceosidin were isolated from all of the species. In addition, isoschaftoside and isovitexin were isolated from *C. virgata*; hispidulin, eupatorin, nepetin, kaempferol 3-*O*-glucoside, and kaempferol 3-methyl ether from *C. virgata* and *C. inermis*; but only 6-methoxyluteolin 3′,4′,7-trimethyl ether from *C. kilea*.

All compounds were tested for their antibacterial activity by disc-diffusion methods (4, 5). None of them showed inhibitory effect on *Staphylococcus aureus* and *Staphylococcus epidermidis*. Only five of the flavonoids showed activity against the bacterial species given in Table 1, and this was at a low level with the most active being apigenin.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Spectra were recorded with the following instruments: uv, Varian Techtron model 635; pmr, Varian 90 MHz; ms, Dupont 21-491 Instrument. Adsorbants for tlc and cc were from Merck. Sephadex was from Pharmacia. Nutrient broth and agar were from Difco.

PLANT MATERIALS.—The plant materials were collected from several parts of Turkey. Voucher specimens for *C. virgata* (no. 45511, from Eskişehir), *C. kilea* (no. 45854, from Kırklareli), and *C. inermis* (no. 48868, from Istanbul) are deposited in the Herbarium of the Faculty of Pharmacy, University of Istanbul.

TABLE 1. Antibacterial Activity of the Flavonoids on Standard Bacterial Strains

Substances	Bacteria									
	Bacillus subtilis ATCC 6633		*Klebsiella pneumoniae* UJ 57		*Proteus vulgaris* ATCC 8427		*Pseudomonas aeruginosa* ATCC 1539		*Escherichia coli* ATCC 8739	
	MIC (μg/ml)	MBC (μg/ml)	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
6-Methoxyapigenin	125	>250	—	—	125	>250	—	—	—	—
Apigenin	109.5	219	219	438	54.8	438	109.5	438	109.5	438
6-Methoxyluteolin 4′,7-dimethyl ether	93.8	187.5	—	—	—	—	—	—	187.5	>375
6-Methoxyluteolin 3′-methyl ether	—	—	—	—	—	—	—	—	125	250
6-Methoxyluteolin 3′,4′,7-trimethyl ether	—	—	—	—	—	—	125	250	250	500

EXTRACTION AND SEPARATION.—Air-dried and powdered aerial parts of *C. virgata* (500 g), *C. kilea* (700 g), and *C. inermis* (500 g) were extracted with EtOH (80%) and worked up by standard procedures (6). After evaporation, each concentrate was reextracted with *n*-hexane, $CHCl_3$, and EtOAc. Two-dimensional paper chromatography showed the flavonoids in $CHCl_3$ and EtOAc phases, and only in the case of *C. virgata* did the remaining aqueous phase also contain flavonoids, in addition to the above-mentioned phases. These extracts were separately chromatographed on polyclar columns, and the single compounds obtained were purified on Sephadex LH 20 with MeOH.

STRUCTURAL DETERMINATIONS.—Structures were determined by spectral methods (uv, pmr) as well as by authentic samples comparison. A diazotized benzidine reagent was used to distinguish 6-*C*-glycosyl and 6,8-di*C*-glycosyl flavonoids; while the former gave a red color, yellow was observed with the latter.

ANTIBACTERIAL ACTIVITY.—Paper discs impregnated with sample solutions were placed on nutrient agar plates, and samples which had zones of more than 7 mm inhibition were selected for the tube-dilution test (7). The lowest concentration of sample required to inhibit the growth of the test organism was designated as the minimum inhibitory concentration (MIC), and the lowest concentration of sample at which the subculture from the test dilution yielded no viable organisms was recorded as the minimum bactericidal concentration (MBC).

ACKNOWLEDGMENTS

This study was supported by TUBITAK (Scientific and Technical Research Council of Turkey, Grant TBAG/579). The authors would like to thank Prof. T.J. Mabry (Austin, Texas) for pmr spectra.

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Received 7 October 1983