were used: (*a*) chloroform–acetone–ethanol (70:30:3 v/v); (*b*) cyclohexane–ethyl acetate–methanol–water (100:100:60:8 v/v); and (*c*) benzene–acetic acid (95:5 v/v). After each development, the plates were dried at 110° and spots were visualized in iodine vapors. The chromatograms corresponded perfectly in color (brown) and R_f values to those obtained with the reference compounds.

Comparison of HPLC, GLC, and UV Methods—The proposed HPLC method was compared to GLC (5) and UV spectrophotometric (2) assays. The results (Tables I–III) show that the proposed method is in good agreement with the GLC and UV assays.

The HPLC procedure described here provides a rapid, sensitive (the lower sensitivity limit is about 10 ng on the column), and precise assay of feprazone in pharmaceutical formulations.

REFERENCES

(1) R. Perego, A. Gallazzi, P. C. Vanoni, and I. Lucarella, *Arzneim.-Forsch.*, **22**, 177 (1972).

(2) G. Coppi, G. Bonardi, and R. Perego, *ibid.*, **22**, 234 (1972).

(3) C. Schutz and H. Schutz, *ibid.*, **23**, 428 (1973).

(4) G. Coppi, A. Vidi, A. Gallazzi, and R. Perego, *ibid.*, **24**, 801 (1974).

(5) A. Gallazzi and P. C. Vanoni, *Il Farmaco, Ed. Pratica*, **30**, 147 (1975).

(6) D. J. Berry, *J. Chromatogr.*, **144**, 159 (1975).

(7) H. G. Dean, B. Donovan, and P. Rylett, *Arzneim.-Forsch.*, **26**, 1574 (1976).

(8) A. Bonora and P. A. Borea, *Experientia*, **34**, 1486 (1978).

GLC–Mass Spectrometry of *Teucrium polium* Oil

M. M. A. HASSAN ˣ, F. J. MUHTADI, and A. A. AL-BADR

Received June 6, 1978, from the *Faculty of Pharmacy, University of Riyad, Riyad, Saudi Arabia.* Accepted for publication December 14, 1978.

Abstract □ The essential oil of *Teucrium polium*, growing in Saudi Arabia, was thoroughly investigated for its constituents by GLC–mass spectrometry, TLC, and spectrophotometric methods. This investigation revealed the presence of 10 terpenoidal compounds including the hydrocarbons β-pinene, limonene, α-phellandrene, and γ- and δ-cadinenes and the alcohols linalool, terpine-4-ol, cedrol, cedrenol, and guaiol. The oil was rich in alcohols and devoid of esters. Preliminary pharmacological screening showed that the oil possesses powerful antispasmodic activity.

Keyphrases □ GLC–mass spectrometry—analysis, *Teucrium polium* oil, antispasmodics, folk medicine □ *Teucrium polium*—oil, GLC–mass spectrometry □ Antispasmodics—analysis, *Teucrium polium* oil, GLC–mass spectrometry, folk medicine □ Folk medicine—antispasmodics, *Teucrium polium* oil, GLC–mass spectrometry

Teucrium polium (Family Labiatae) is one of the most fragrant plants in Saudi Arabia and is fairly distributed throughout the country (1). In folk medicine, it is used as an antispasmodic, antirheumatic, carminative, and flavoring agent. In an admixture with other powdered herbs, it is claimed to be therapeutic for peptic ulcer.

The aroma of this plant is due to its essential oil content (2). A literature review revealed that the oil constituents had not been investigated thoroughly (4). The present work was carried out to investigate the composition of this potentially therapeutic plant oil.

EXPERIMENTAL

Material—The plant was collected in the spring at the flowering stage from Sudair (Central Zone), 180 km from Riyad. Its identity as *T. polium* was confirmed[1]. It was dried and powdered.

Successive quantities (1 kg each) were subjected to steam distillation, which produced a volatile oil (average yield, 1.55 g).

The physical properties of the oil are presented in Table I.

TLC—TLC was done on precoated silica gel thin-layer plates[2] developed in chloroform–benzene (1:1) and chloroform–benzene (4:1) solvent systems (3). After development, the spots were visualized by spraying with anisaldehyde reagent (3).

Table I—Comparative Physicochemical Data

Property	Isolated Oil	Sardinian Oil
Color	Light yellow, darkens upon standing	Blue
Odor	Fragrant, characteristic	Agreeable, pungent
Refractive index[a]	n_D^{24}, 1.4850	n_D^{20}, 1.475 and 1.488
Optical rotation[b]	$[\alpha]_D^{24}$ +4.7° (10% in ethanol)	$[\alpha]_D^{20}$ +12.75°
Acetyl value[c]	117	47.45–47.59
IR[d]	1633, 1655, 1700, 2900, and 3400 cm^{-1}	—
NMR[e]	Broad singlets at 332, 306, 280, and 268 Hz; broad singlet at 217 Hz; singlet at 98 Hz; doublet at 73 Hz; two overlapping doublets centered at 58 Hz	—

[a] Zeiss Abbe refractometer. [b] Bellingham and Stanley polarimeter. [c] According to the BP method (5). [d] As film on Perkin-Elmer 567 grating IR spectrophotometer. [e] In deuterochloroform on Varian T 60A with tetramethylsilane as the internal standard.

GLC–Mass Spectrometry—Mass spectra of the volatile oil constituents were obtained using a gas chromatograph–mass spectrometer[3]. The chromatographic column (1.8 m long) was packed with 3% OV-17. The column was conditioned isothermally at 100° for 10 min, and then the temperature was programmed at 5 min until 260°. The injection port temperature was 185°, the separation temperature was 252°, and the ion source temperature was 290°. Mass spectra were obtained by operating at 60 MHz and with an ionization energy of 70 ev.

RESULTS AND DISCUSSION

The isolated oil was compared with Saridinian *T. polium* oil (4) (Table I). The IR and NMR data revealed free alcohols (3400 cm^{-1}; broad singlet at 217 Hz, disappeared on deuteration), alkene and highly conjugated

[1] The plant material was identified as *Teucrium polium* Linneus (Labiatae) by Dr. A. M. Migahid, Department of Botany, Faculty of Science, University of Riyad, Riyad, Saudi Arabia. A voucher specimen is available in the herbarium of Riyad University.

[2] GF plants, Anachem.

[3] Model 9000, LKB-Produkter, Brommo, Sweden.

0022-3549/79/0600-0800$01.00/0

Table II—Constituents of the Oil by GLC-Mass Spectrometry

Compound	Retention Time, min	Molecular Weight	M^+
1 β-Pinene	1.0	136.23	136
2 Limonene	1.3	136.23	136
3 α-Phellandrene	1.6	136.23	136
4 Linalool	2.3	154.24	136
5 Terpine-4-ol	3.9	154.24	154
6 γ-Cadinene	17.2	204.34	204
7 Cedrenol	20.7	222.36	220
8 δ-Cadinene	22.5	204.34	204
9 Guaiol	22.9	222.36	222
10 Cedrol	23.4	222.36	222

alkenes [1633 (m)[4] and 1655 (m) cm^{-1}; broad peaks at 332, 306, 280, 268 Hz], alicyclic structures (singlet at 98 Hz, doublet at 73 Hz, and triplet formed of two overlapping doublets at 58 Hz), and the absence of esters, other carbonyl compounds, and aromatic functions. TLC screening of the oil showed both hydrocarbons and alcohols.

GLC–mass spectrometry studies indicated the presence of several compounds (Table II and Fig. 1). The major components on the GLC trace (Fig. 1) are identified by sequence numbers that correspond to the mass spectral sequence numbers.

The mass spectra so obtained were compared with the authentic compounds and demonstrated almost identical fragmentation characteristics (6). The mass fragmentation pattern of most components showed molecular ion peaks at atomic mass units equivalent to their molecular weights. The linalool mass fragmentation pattern exhibited an M^+ at 136 amu instead of at 154 amu. The M − 18 fragment was attributed to the formation of a triene compound from the parent ion by the loss of water (7).

The cedrenol mass spectrum did not show an M^+ at 222 amu; the M − 2 fragment at 220 amu represented the loss of two hydrogen atoms (7).

From the data obtained, the volatile oil was considered to be one of the alcoholic volatile oils since 50% of the components were alcohols. The absence of esters may indicate that the plant is unable to esterify these alcohols.

Preliminary pharmacological screening of the isolated oil showed that it possessed potent antispasmodic activity and that it markedly antagonized the effects of the agonists nicotine, acetylcholine, histamine, and serotonin.

[4] Medium.

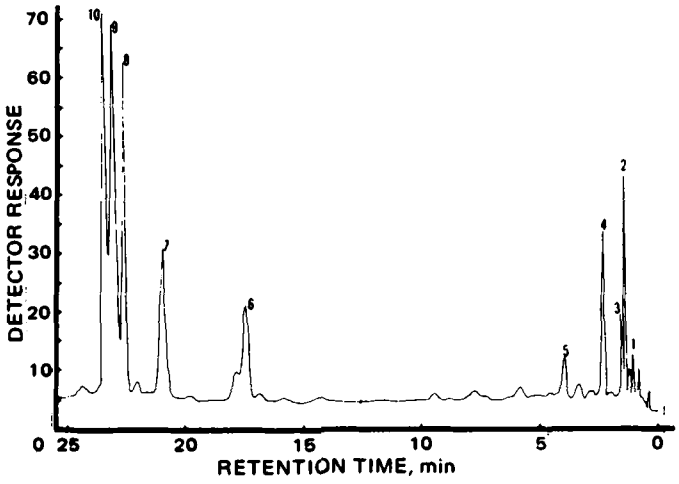

Figure 1—*Gas chromatogram of the isolated oil.*

The aqueous extract of the herb had low antispasmodic activity compared to the oil. The results of this study partially justified the use of this plant in folk medicine.

REFERENCES

(1) A. M. Migahid and M. A. Hammouda, "Flora of Saudi Arabia," Riyad University Publications, Riyad, Saudi Arabia, 1974.

(2) G. M. Wassel and S. S. Ahmed, *Pharmazie*, **29**, 8 (1974).

(3) E. Stahl, "Thin Layer Chromatography," 2nd ed., Allen & Unwin, London, England, 1967, p. 857.

(4) I. E. Puxeddu, *Ann. Chim. Appl.*, **15**, 159 (1925).

(5) "British Pharmacopoeia, 1973," Her Majesty's Stationery Office, London, England, 1973.

(6) E. Stenhagen, S. Abrahamasson, and F. W. McLafferty, "Registry of Mass Spectra Data," vols. I and II, Wiley, New York, N.Y., 1974.

(7) H. Budzikiewicz, C. Djerassi, and D. H. William, "Interpretation of Mass Spectra of Organic Compounds," Holden-Day, San Francisco, Calif., 1965, pp. 32, 33.

ACKNOWLEDGMENTS

The authors thank The Upjohn Co., Kalamazoo, Mich., for the GLC–mass spectrometric data and A. M. S. Ahmed for assistance.