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MARRUBIOL—A NEW DITERPENOID FROM MARRUBIUM VULGARE

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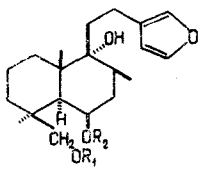
The furan diterpenoid marrubiin has been isolated from the plant Marrubium vulgare L. (common hoarhound), family Labiatae and has been studied [1–3]. Recently, papers have been published on the biosynthesis of this diterpenoid [4]. We have made a detailed investigation of an extract of the epigeal part of the plant in order to investigate secondary metabolites possibly having a biogenetic relationship with marrubiin.

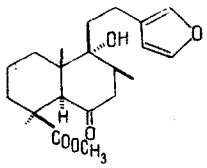
From an acetone extract of the plant collected in the northern regions of the Moldavian SSR, after the far-reaching separation of marrubiin and the acid fraction with subsequent chromatography on alumina and silica gel, we obtained an additional amount of marrubiin, β-sitosterol, the diterpene alcohol peregrinol, which has been found previously in another species of Marrubium [5, 6], and also two new diterpenoids which we have called marrubiol and vulgarol.

Below we give the R_f values of the substances from Marrubium vulgare on chromatography in a thin fixed layer of silica gel in the benzene—acetone (17: 3) system; five spots were found after treatment of the plate with concentrated sulfuric acid and subsequent heating.

Substance	R_f
Marrubiin	0.58
β-Sitosterol	0.53
Peregrinol	0.46
Marrubiol	0.26
Vulgarol	0.22

Marrubiol, $C_{20}H_{32}O_4$ (I), according to spectral data, is a furan alcohol. On reaction with acetic anydride in pyridine it readily forms a monacetate (II), the oxidation of which with chromic acid gives a hydroxy ketone (III).





I. $R_1 = R_2 = H$ II. $R_1 = COCH_3, R_2 = H$ IV

III. $R_1 = COCH_3$ $-OR_2 = O$

The above facts, and also the IR spectrum of (I) show that marrubiol is a triol containing primary, secondary, and tertiary hydroxy grups. In view of the fact that the IR and UV spectra of marrubiol are very similar to the spectra of some derivatives of marrubiin, we concluded that this triol is reduced marrubiin. In actual fact, the reaction of the latter with lithium aluminum hydride in tetrahydrofuran gave a triol which was identical in respect of melting point, results of thin-layer chromatography, IR spectra, and specific rotation with marrubiol. Consequently the structure and stereochemistry of marrubiin may be expressed by formula (I). However, when the keto ester (III) synthesized from marrubic acid was reduced within lithium aluminum hydride, a triol was obtained the IR spectrum of which was identical with that of (I) but which had $[\alpha]_D^{20}$ + 29.4° [for (I) $[\alpha]_D^{20}$ + 12.5°]. This is evidently connected with the epimerization of the C_6 center in the reduction of the keto group. The relatively low polarity of (I) as compared with some diols (for example, vulgarol) is due to the strong association of the hydroxy groups at C_{18} and C_6, which can also be seen from the IR spectrum (intramolecular associate at 3220 cm^{-1}). The combined presence in the plant of marrubiol and marrubiin gives grounds for assuming that the former is a direct precursor of the latter.

The other diterpenoid—vulgarol, $C_{20}H_{36}O_2$—is, according to its IR spectrum, a diol with one trisubstituted double bond and a readily acetylated hydroxy group. The study of its structure is continuing.

Experimental

The IR spectra were taken on a UR-10 spectrometer, the melting points were determined on a Kofler block without correction, and $[\alpha]_D$ values were measured in chloroform.

An acetonic extract (11 kg) of the air-dried plant was evaporated to a volume of 2 *l*, the marrubiin that separated as a precipitate (15 g) was separated off, and the filtrate was evaporated to dryness. The residue was dissolved with heating in 1.5 *l* of methanol. After cooling, the wax that deposited was separated off, and the methanol was distilled off.

The resulting residue was dissolved in 3 *l* of ether and washed several times with 5% caustic soda solution. The ethereal layer was washed with water, dried with sodium sulfate, and distilled. The extracts purified in this way (60 g) were chromatographed on 2 kg of alumina (activity grade III). The fractions were checked by thin-layer chromatography in a nonfixed layer of alumina and in a fixed layer of silica gel. Benzene (1.5 *l*) eluted a fraction containing β-sitosterol (3 g), mp 137° C (from methanol); a mixture with an authentic sample gave no depression of the melting point, and their IR spectra were also identical. A mixture of benzene and ether (9:1 and 4:1, 5 *l*) eluated 3 g of marrubiin. After three crystallizations from ethanol, mp 158–160° C, giving no depression of the melting point with an authentic sample of marrubiin; their IR spectra were also identical.

The fractions eluted by the first portions of ether (0.7 *l*) contained peregrinol. After purification on a column of activated carbon in acetone and three crystallizations from a mixture of petroleum ether and benzene, 400 mg of pure peregrinol was obtained. The melting point of the substance and of a mixture with an authentic sample were 129–130° C, and the IR spectrum was identical with that of the peregrinol described previously [6].

From the fractions obtained on subsequent elution with ether (2.2 *l*), rechromatography on 40 g of silica gel (benzene–ether (2:1)) yielded marrubiol (1.5 g) in the form of a viscous liquid. On drying in vacuum, it was converted into an amorphous powder with a softening point of about 60° C, $[\alpha]_D^{20}$ + 12.5° (c 7.2). IR spectrum (in CCl_4) 3625, 3620, 3220, 1160, 1070, 1035, 1020 (hydroxy groups), 1560, 1505, 878 cm^{-1} (furan); λ_{max} (in ethanol) 213 mμ (log ε 3.60).

Found, %: C 71.61, 71.31; H 10.01, 10.01. Calculated for $C_{20}H_{32}O_4$, %: C 71.39; H 9.59.

On prolonged storage, the substance decomposed with the formation of a dark brown liquid.

Fractions eluted with 2 *l* of a mixture of ether and ethanol (20:1) were purified by the filtration of an acetonic solution through 5 g of activated carbon and were chromatographed on 50 g of silica gel. A mixture of benzene and ether (1:1) eluted 350 mg of vulgarol. After crystallization from a mixture of petroleum ether and benzene and drying in vacuum, mp 152–153° C, $[\alpha]_D^{20}$ ± 0° (c 6.5). IR spectrum (KBr tablet): 3330, 1120, 1000 (hydroxy groups), 1670, 860 cm^{-1} (trisubstituted double bond).

Found, %: C 77.75, 77.55; H 11.82, 11.55. Calculated for $C_{20}H_{36}O_2$, %: C 77.86; H 11.76.

Acetylation of marrubiol. A solution of 100 mg of marrubiol in 3 ml of dry pyridine was treated with 1 ml of acetic anhydride, and the mixture was left at room temperature for 16 hr. Then it was diluted with water and extracted with ether, the ethereal solution was washed with 10% hydrochloric acid and with water and was dried with magnesium sulfate, and the solvent was distilled off. The residue was chromatographed on 10 g of silica gel. A mixture of benzene and ether (3:1) eluted 90 mg of acetate in the form of a viscous liquid. IR spectrum (film): 3530, 1160, 1065, 1030 (hydroxy groups), 1725, 1245 (acetate), 1570, 1505, 880 cm^{-1} (furan).

Found, %: C 69.95, 69.88; H 9.35, 9.10. Calculated for $C_{22}H_{34}O_5$, %: C 69.81; H 9.05.

Oxidation of marrubiol acetate. A solution of 70 mg of the substance in 1 ml of pyridine was added in drops to the complex prepared from 200 mg of chromic anhydride and 2 ml of pyridine. The mixture was kept at room temperature for 18 hr. After the usual working up, the product was chromatographed on 5 g of silica gel. A mixture of benzene and ether (3:1) eluted the pure keto acetate (III) in the form of a viscous liquid (60 mg). IR spectrum (film): 3540, 1160 (hydroxyl), 1735, 1240 (acetate), 1710 (ketone), 1563, 1505, 878 cm^{-1} (furan).

Reduction of marrubiin with lithium aluminum hydride. A mixture of 150 mg of marrubiin, 7 ml of tetrahydrofuran, and 100 ml of lithium aluminum hydride was boiled for 5 hr. The excess hydride was decomposed, the solution was acidified, and the substance was extracted with ether. The residue after the distillation of the ether was chromatographed on 5 g of silica gel. Elution with a mixture of benzene and ether (2:1) gave 110 mg of a viscous liquid which, on drying in vacuum, was converted into an amorphous mass melting at about 60° C, $[\alpha]_D^{20}$ + 13.2° (c 8.3). The IR spectrum was identical with that of marrubiol.

Preparation of the keto ester (III). The methyl ester (250 mg) obtained by the methylation of marrubic acid with diazomethane was oxidized with chromic anhydride (200 mg) in pyridine at room temperature for 20 hr: This yielded 220 mg of the keto ester with mp 121–123° C (petroleum ether–benzene), IR spectrum (KBr tablet): 3560, 1165, (hydroxyl), 1725, 1225 (ester), 1705 (ketone), 1580, 1505, 878 cm^{-1} (furan).

Found, %: C 69.72, 69.70; H 8.32, 8.28. Calculated for $C_{21}H_{30}O_5$, %: C 69.58; H 8.34.

Reduction of the keto ester (III). A solution of 70 mg of the substance in 5 ml of tetrahydrofuran and 50 ml of lithium aluminum hydride was boiled for 2 hr. After the usual treatment, 55 mg of a liquid product was obtained the IR spectrum of which was identical with that of marrubiol, $[\alpha]_D^{20}$ + 29.4° (c 6.5).

Conclusions

1. Peregrinol and two new diterpenoids—marrubiol and vulgarol—have been isolated from the plant Marrubium vulgare L. for the first time.

2. The structure of marrubiol (I), which is possibly a biogenetic precursor of marrubiin, has been demonstrated.

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