A CHEMICAL INVESTIGATION OF *Achillea nobilis*

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From the flower heads and leaves of *Achillea nobilis* collected in the flowering phase close to Balytkykol, Egindybulak region, Karaganda Province, Kazakh SSR, by extraction with chloroform and chromatography of the combined substances on a column of silica gel we have isolated a new sesquiterpene lactone anobin $C_{15}H_{20}O_5$, mp 175.5-177.5°C and have identified for the first time estafiatin, $C_{15}H_{18}O_3$, mp 102-104°C $[\alpha]^{20}$ −10.3° (chloroform); hanphyllin, $C_{15}H_{20}O_3$, mp 189°C (decomp), $[\alpha]_D^{20}$ + 58.6° (c 0.39; chloroform), and 3,5-dihydroxy-6,7,8-trimethoxyflavone, $C_{18}H_{16}O_7$, mp 148-150°C. On the basis of chemical and spectral characteristics it has been established that anobin has the structure of 4α,10α-dihydroxy-2α,3α-epoxy-5,7α(H),6β(H)-guai-11(13)-en-6,12-olide. It has been established that estafiatin possesses a pronounced growth regulating activity.

We have established previously that total extractive substances from *Achillea nobilis* L. inhibit the germination of the seeds of certain plants [1].

From the epigeal part of *Achillea nobilis* collected in the flowering phase in the Egindybulak region of Karaganda province, KazakhSSR, by extraction with chloroform and the chromatography of the total substances on a column of silica gel, we have isolated four crystalline substances.

Substance (I) — $C_{15}H_{20}O_5$, mp 175-5.177.5°C (from ethanol) — proved to be a new sesquiterpene lactone and we have called it anobin. IR spectrum (cm^{-1}: ν_{max} 3500 (OH group), 1760 (γ-lactone carbonyl), 1660 (C=C).

The PMR spectrum of anobin showed singlets at 1.16 and 1.56 ppm (3 H each) of tertiary methyls, weakly resolved doublets at 2.23 and 3.44 ppm (1 H, each, J = 2 Hz for each) of protons at an epoxy group, a multiplet in the 3.9-ppm region (1 H) of H_7, a triplet at 4.38 ppm (1 H, J = 12.4 Hz), of a lactone proton, a singlet at 4.93 ppm (2 H), of the protons of hydroxy groups, and doublets in the 5.28- and 6.10-ppm region (1 H each, J = 2.4 Hz) of the protons of an exomethylene group conjugated with a carbonyl group. The H_1 and H_5 protons appear in the form of singlets at 2.70 and 2.76 ppm ($J_{1,5}$ = 0).

The hydroxy groups were not acetylated by acetic anhydride in pyridine and were not oxidized by chromium trioxide, i.e., they were tertiary.

With the presence of twenty hydrogen atoms, an epoxide ring, and two tertiary groups a guaiane structure is characteristic for anobin, as was also shown by the formation of chamazulene on dehydrogenation.

The triplet nature of the signal of the lactone proton in the PMR spectrum showed that the lactone ring was located at C_6–C_7. The large coupling constant of the H_6 and H_5 protons (J = 12.4 Hz) showed their mutual trans position.

A comparison with the IR spectra of sesquiterpene lactones with similar structure (canin and rupins A and B [2]) has permitted the proposal of the α-orientation relative to the main carbon skeleton for the epoxide ring and the hydroxy groups of anobin.

The intensity of the peak of the molecular ion of anobin at m/z 280 was slight in comparison with the main peaks of the mass spectrum, which is obviously due to the relative instability of the guaiane skeleton, intensified because of the presence of epoxy and hydroxy groups. Fragments with m/z 216, 246, 250, and 262 characterized the initial act of the frag-

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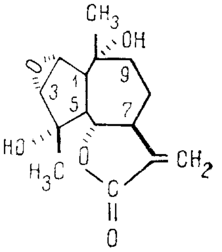
mentation of the molecular ion with the splitting out of methyls and hydroxyls in different sequences. The ejection of the neutral particles CH_3OH formed an ion with m/z 216 and then the breakdown of the main carbon skeleton took place as was confirmed by the presence of an ion with m/z 123. Another direction for the appearance of the ion with m/z 123 was the elimination of an H_2O molecule from the M^+ ion with the participation of the hydroxyl at C_4 and the localization of the charge on the oxygen of the 2,3-epoxy group.

The presence of a peak at m/z 250 can be explained as the result of the simultaneous ejection of methyls geminal to hydroxy groups with the subsequent formation of an ion having m/z 97, and the simultaneous ejection of the OH groups from the M^+ ion led to the appearance of a fragment with m/z 95.

Thus, on the basis of its physicochemical constants, its IR, PMR, and mass spectra, and its chemical transformations it was concluded that anobin has the structure and configuration of 4α,10α-dihydroxy-2α,3α-epoxy-5,7α(H),6β(H)-guai-11(13)-en-6,12-olide (I).

Substance (II) — $C_{15}H_{18}O_3$, mp 102-104°C (ether), $[\alpha]_D^{20}$ −10.3° (chloroform). The IR spectrum showed absorption bands at (cm^{-1}) 1760 (γ-lactone C=O), 1670 and 1650 (C=C). In the PMR spectrum there were singlets in the 1.53-ppm region (3 H, methyl, geminal to oxygen), and at 3.28 ppm (1 H, H_3), a triplet at 4.01 ppm (1 H, $J_1 = J_2 = 10$ Hz; lactone proton), singlets of one proton unit each at 4.78 and 4.87 ppm (protons of an exocyclic vinyl group), and doublets in the 5.42- and 6.12-ppm regions (1 H, J = 4 Hz, the protons of an exocyclic methylene group conjugated with a carbonyl group).



By a comparison of the physicochemical constants obtained and spectral characteristics with the literature [3], substance (II) was identified as the guaianolide estafiatin. This is the first time that estafiatin has been isolated from *Achillea nobilis*.

In a study of growth-regulating action it was established that estafiatin at a concentration of $4 \cdot 10^{-3}$ M inhibited the germination of summer cypress seeds by 49.2% and suppressed the germination of amaranth seeds completely. At $2 \cdot 10^{-2}$ M this lactone retarded the germination of mustard seeds by 73.6% and of summer cypress by 90.4% while wild oats seeds lost their germinating capacity completely. At a concentration of $4 \cdot 10^{-4}$ to $4 \cdot 10^{-3}$ M estafiatin inhibited the growth of wheat coleoptiles of the Saratovskaya 29 variety by 75-92%.

Substance (III) — $C_{15}H_{20}O_3$, mp 189°C (decomp., ethanol), $[\alpha]_D^{20}$ +58.6° (c 0.39; chloroform). Its IR spectrum had absorption bands at (cm^{-1}) 3490 (OH group), 1740 (γ-lactone carbonyl), and 1660 (C=C). The PMR spectrum showed the signals of methyls at a double bond — singlets in the 1.40-ppm (3 H) and 1.64-ppm (3 H) regions, of a hemihydroxylic proton at 4.12 ppm (1 H), of lactone and olefinic protons in the 4.84-ppm (2 H) and 4.92-ppm (1 H) regions, and of the proton of a hydroxy group in the form of a weakly split doublet of one proton unit at 5.24 ppm, the protons of an exocyclic methylene group conjugated with a carbonyl γ-lactone being shown by doublets at 5.75 and 6.15 ppm (1 H each).

The ^{13}C NMR spectrum contained two quartets ($-CH_3$): 11.84 and 15.91 ppm; four triplets ($>CH_2$): 27.36, 35.43, 40.44, and 119.46 ppm; five doublets ($-\overset{|}{\underset{|}{C}}H$): at 49.23, 76.79, 81.03, 123.41, and 125.13 ppm, and four singlets ($-\overset{|}{\underset{|}{C}}-$): at 136.86, 140.35, 143.44, and 169.87 ppm, which confirms the deductions from the PMR spectrum and makes it possible to assign this compound to the germacranolides with two methyl groups, an exomethylene group conjugated with the lactone carbonyl, a hydroxy group, and two double bonds in the carbon skeleton.

When this substance was acetylated with acetic anhydride in pyridine a derivative with the composition $C_{17}H_{22}O_4$, mp 240-241°C (ethanol) was obtained the IR spectrum of which showed the absence of hydroxy groups and the appearance of absorption bands characteristic for an ester group at 1735 and 1270 cm^{-1}. The PMR spectrum of the acetyl derivative had the signals of methyls at a double bond — singlets at 1.43 and 1.68 ppm (3 H each); the methyl of an acetyl group — singlet at 2.08 ppm (3 H); that of a lactone proton — quartet at 4.55 ppm (1 H, J_1 = 10 Hz and J_2 = 8.7 Hz), of olefinic protons — doublets at 4.86 and 4.98 ppm; of a hemiacetyl proton — doublet of doublets at 5.15 ppm, and of the protons of an exomethylene group conjugated with the carbonyl of a lactone ring — doublets at 5.52 and 6.26 ppm.

On the basis of the IR, PMR, and ^{13}C spectra and from the results of chemical transformations, substance (III) was identified as the germacranolide hanphyllin [4]. This is the first time that hanphyllin has been isolated from *Achillea nobilis*.

Substance (IV) — $C_{18}H_{16}O_7$, mp 148-150°C (ethyl acetate), ν_{CO} 1650 cm^{-1}, $\lambda_{max}^{C_2H_5OH}$ 239, 258, 274, 348 nm. The PMR spectrum showed the singlets of three CH_3O groups (3.832 ppm, 3 H, 3.878 ppm, 3 H; 3.937 ppm, 3 H) and of two OH groups (12.54 ppm, 2 H), and the signals of five aromatic protons (6.428, 1 H; 6.950, 2 H, 7.626, 2 H) which is characteristic for an unsubstituted B ring. Acetylation gave a diacetate $C_{22}H_{20}O_9$ with mp 158-160°C (ethyl acetate), ν_{CO} 1625, 1755 cm^{-1}.

Thus, substance (IV) has the structure of 3,5-dihydroxy-6,7,8-trimethoxyflavone [5].

EXPERIMENTAL

The individuality of the substances was checked by thin-layer chromatography (TLC) on Silufol plates in the benzene—ethanol (9:1) system. The revealing agents were iodine vapor and 0.5% $KMnO_4$ in 0.5% H_2SO_4. The substances for analysis were dried in a vacuum pistol with P_2O_5 over ethanol for 6-8 h.

Specific optical rotations were measured on a Zeiss polarimeter in a tube 0.5 dm long with a volume of 1 ml.

IR spectra (tablets with KBr) were taken on a UR-20 spectrometer, UV spectra on a Specord UV-Vis, PMR spectra on a Bruker HX-90E (in deuterochloroform and deuteropyridine, 0 — TMS, δ scale), ^{13}C PMR spectra on a Bruker HX-90 pulsed spectrometer (22.63 MHz, $CDCl_3$, 0 — TMS), and mass spectra on a Varian CH-8 at 70 V with recording temperature of 90 and 100°C.

The results of elementary analysis of the compounds agreed with the calculated figures.

Isolation of the Total Substances. The flower heads and leaves of *Achillea nobilis* (8.3 kg) were exhaustively extracted with chloroform. After the chloroform had been distilled off, 212 g of resin was dissolved in 350 ml of ethanol at room temperature, and the solution was diluted 2:1 with hot water. After a day, the precipitate was separated off and the aqueous alcoholic solution was treated with chloroform. The chloroform extracts were concentrated, and the resin (74 g) was chromatographed on KSK silica gel at a ratio of material to support of 1:15. The column was eluted with benzene, with benzene—ether mixture 4:1.3:2.1:1 with ether, ether—ethyl acetate 3:2.1:1, and with ethyl acetate. Fractions with a volume of 300-400 ml were collected.

Isolation of Anobin. The benzene fractions deposited colorless rhombic crystals which on TLC gave a single spot with Rf 0.37; they were recrystallized from ethanol and dried over P_2O_5 in vacuum at 76°C. This gave a substance with the composition $C_{15}H_{20}O_5$, mp 175.5-177.5°C.

Dehydrogenation of Anobin. The structure (0.160 g) was dehydrogenated over selenium (0.08 g) at 280-300°C for 10 min. The product was extracted with hexane and was chromatographed on a column of Al_2O_3 (activity grade II). Hexane eluted a blue oil, $C_{14}H_{16}O$, bp 158-160°C/8 mm, d_4^{20} 0.9883, which was identified as chamazulene.

Isolation of Estafiatin. The benzene—ether (4:1) fractions yielded colorless acicular crystals with Rf 0.50, which were recrystallized from ether and dried over P_2O_5 at 76°C (10 mm Hg). The substance had the composition $C_{15}H_{18}O_3$, mp 102-104°C.

Isolation of Hanphyllin. When the column was eluted with benzene—ether (1:1), colorless lamellar crystals with Rf 0.30 were isolated. After recrystallization from ethanol, a crystalline substance with the composition $C_{15}H_{20}O_3$ was obtained. In a determination of the melting point on a metal block, it underwent a transformation at 167°C and then melted at 189°C.

Acetylation of Hanphyllin. To 0.2 g of the crystalline substance in 4 ml of pyridine was added 2 ml of acetic anhydride. The mixture was heated in the water bath at 50-60°C for 2 h. After cooling, it was diluted with 90 ml of H_2O and extracted three times with 100-ml portions of $CHCl_3$. The chloroform extracts were washed with 3% HCl. After the solvent had been driven off, an oily product was obtained to which an excess of ether was added. The resulting precipitate was separated off and was recrystallized three times from ethanol. This gave a colorless crystalline substance with the composition $C_{17}H_{22}O_4$, mp 240-241°C.

Isolation of the Flavonoid Component. When the elution of the column containing the total substance was continued with ether—ethyl acetate (1:1) and with ethyl acetate, yellow crystals were isolated. Repeated purification by ether and recrystallization from ethyl acetate yielded a yellow crystalline substance with the composition $C_{18}H_{16}O_7$, mp 148-150°C, R_f 0.26.

CONCLUSIONS

1. A new sesquiterpene lactone, anobin, has been isolated from the epigeal part of *Achillea nobilis* L., and estafiatin, hanphyllin, and 3,5-dihydroxy-6,7,8-trimethoxyflavone have been identified in it for the first time.

2. On the basis of chemical and spectral characteristics it has been established that anobin has the structure of 4α,10α-dihydroxy-2α,3α-epoxy-5,7α(H),6β(H)-guai-11(13)-en-6,12-olide.

3. It has been established that estafiatin possesses a pronounced growth-regulating activity: It inhibits the germination of mustard, summer cypress, amaranth, and wild oats and the growth of wheat coleoptiles.

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