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THE LEAF OILS OF WASHINGTON CONIFERS: II. JUNIPERUS SCOPULORUM.*

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In a previous paper, a review was made of work which has been done on conifers of Washington. The present paper deals with the Rocky Mountain juniper. It does not occur to a great extent, being limited to certain regions, but it happened to be easily available to the writers and forms the basis of this investigation.

This species of juniper is a tree, ten to forty feet in height, with a short stout trunk, two feet or more in diameter, and often divided near the ground into a number of slightly spreading stems. The stout, spreading branches, which are covered with a scale-like bark, give the tree a rounded appearance. The bark of the trunk is reddish brown in color. The slender branches are covered with a thin, scaly bark. The leaves are opposite in pairs, acute, glandular and dark green. The fruit is about one-sixteenth of an inch in length and blue or rose color, ripening at the end of the second season, when it is from one-fourth to one-third of an inch in diameter, bright blue, covered with a glaucous bloom and usually two-seeded. The ripe seed is ovate, acute, light chestnut-brown, lustrous and about three-sixteenths of an inch in length.

Juniperus scopulorum, found ordinarily in arid regions, is common enough east of the Cascades and in the Rocky Mountains, whence its name, Rocky Mountain juniper. Strangely enough it has crossed its natural barrier, the Cascades, to reappear west of these mountains in only a few isolated localities in the northern Puget Sound region where there is an abundance of soil moisture. The material for this work was obtained from trees growing in bottom land having a sandy and clayey composition. This section receives an annual rainfall of about forty inches and, from the general character of the land, the indications are that this area is covered with water during certain times of the year, especially after heavy rains.

EXPERIMENTAL.

The material was gathered in the spring from a grove of trees near Everett, Washington. The leaves were separated as far as possible from the twigs and im-

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mediately distilled with steam. The oil was separated roughly from the aqueous distillate, which was once cohobated. The cohobated liquid was extracted with ether which was then separated and added to the first portion of oil. The ether solution was carefully dried and the solvent was largely removed by distillation, the last portions being separated by means of a vacuum. The total oil thus obtained from 336 pounds of material amounted to 750 cc., a yield of 0.49 per cent.

When freshly distilled, the oil was yellowish green and possessed a pleasing, balsamic odor, somewhat resembling that from blueberries. There appeared to be little tendency to resinification or hydrolysis, and the color showed little change after several months. The general constants were as follows: d_{25}° 0.0966; $[\alpha]_D^{25}$ +41.18°; n_{D20} 1.4856; acid number 1.52; saponification value 11.29. Calculating the ester as the acetate of borneol, the amount is 3.41 per cent, the quantity of alcohol from this by hydrolysis 2.68 per cent.

The oil was first shaken with a 5 per cent solution of sodium carbonate to remove the free acids and then with 5 per cent sodium hydroxide to separate the phenols. The aqueous liquids were then shaken with ether to remove any adhering oil, and the ether solution was added to the residual oil. The ethereal solution was washed with water until the washings were neutral, the latter being added to the sodium hydroxide solution, which was then made acid and completely extracted with ether. After drying with calcium chloride, the ether was evaporated, leaving a small amount of a dark brown oil having a pleasant, creosote-like odor. It did not solidify even after cooling to -15° C. for some time. The amount obtained was too small to permit more than an ultimate analysis.

0.0720 gave 0.1777 CO₂ and 0.0705 H₂O. Found: C, 67.3; H, 10.8. Calculated for C₁₁H₁₆O₂: C, 67.1; H, 10.5.

After drying and removing the ether, the remainder of the oil was fractionated under reduced pressure. That portion which boiled below 100° C. at 10 mm. was purified by repeatedly distilling up to the same temperature, the residue in each case being added to the main body of oil. It was then subjected to fractionation under ordinary pressure and then to several refractionations. Eventually the following portions were collected: 155–160°, 4.2; 160–163°, 18.6; 163–169°, 14.5; 169–174°, 4.3; 174–185°, 3.0 per cent of the total oil.

Alpha Pinene.—The first fraction was a yellow liquid which distilled chiefly at 157° C. An ultimate analysis indicated that it was mixed with a small amount of oxygenated compounds. Attempts to prepare a nitrosochloride were unsuccessful, as might have been expected due to the high optical rotation (6). The presence of α -pinene was readily shown, however, by oxidation to pinonic acid by means of potassium permanganate (4). After completion of the reaction the mixture was distilled with steam to remove excess oil and the residue was filtered, acidified, and completely extracted with chloroform which gave a dark brown, syrupy oil which did not crystallize. A semicarbazone prepared from it was found to melt at 203° C. which identified the oil as pinonic acid.

Camphene.—The second fraction was a colorless liquid which distilled mainly at 160.5–162° C.

0.1235 gave 0.3875 CO₂ and 0.1351 H₂O. Found: C, 87.8; H, 12.1. Calcd. for C₁₁H₁₆: C, 88.2; H, 11.8.

It was hydrated according to Bertram and Walbaum (3) with acetic and sulphuric acids giving a light yellow oil with a camphoraceous odor. At -10° C. a substance crystallized out, but it was found impossible to separate in this way. That hydration had taken place was indicated by reduction in percentage of carbon.

0.1391 gave 0.4209 CO_2 and 0.1405 H_2O . Found: C, 81.8; H, 11.2. Calcd. for $\text{C}_{11}\text{H}_{11}\text{O}$: C, 77.9; H, 11.7.

In order to confirm the presence of isoborneol in this hydrated oil an attempt was made to make its phenyl urethane. The crystalline product, after careful purification, melted at 148° C. and could not be the isoborneol derivative which melts at 138° C. The amount was too small for further investigation. Another portion of the hydrated oil was oxidized with Fittig's chromic acid mixture (1) giving a few oily drops which had a strong odor of camphor.

While no definite evidence could be found of the presence of camphene in the fraction, the authors are convinced of its presence and hope to prove the point later when larger amounts of material are available.

Beta Pinene.—The comparatively large third fraction distilled mainly between 166° and 168° C.

0.1420 gave 0.4576 CO_2 and 0.1537 H_2O . Found: C, 87.9; H, 11.3. Calcd. for $\text{C}_{10}\text{H}_{16}$: C, 88.2; H, 11.8.

The presence of beta pinene was demonstrated by oxidation to nopinic acid with alkaline potassium permanganate (5). The resulting sodium salt was dissolved, and the solution was acidified and extracted with ether, producing a yellow oil which gradually crystallized. From this was separated flat plates which were apparently nopinene glycol $\text{C}_{10}\text{H}_{18}\text{O}$ and a larger amount of needles. After repeated recrystallization from ether the latter were found to melt at 123 – 124° C.

0.1245 gave 0.2848 CO_2 and 0.1011 H_2O ; 0.1038 gave 0.2391 CO_2 and 0.0820 H_2O . Found: C, 62.5 and 62.8; H, 8.8 and 8.6. Calcd. C, 65.2; H, 8.6.

Terpinene.—The fourth fraction which was very small distilled chiefly at 172 – 174° C. The presence of terpinene was readily demonstrated by conversion to the nitrite (2). The product was purified by recrystallization from boiling hydrochloric acid giving fine, white needles melting sharply at 155° C.

The fifth fraction was suspected to contain limonene or dipentene but attempts to prepare derivatives with bromine or with hydrogen chloride gave only uncrystallizable oils.

Saponification and Fractionation.—All of the oil boiling above the terpene fraction was hydrolyzed by heating with alcoholic potash on a water-bath for two hours. After distilling off the greater part of the alcohol, the residue was placed in a separatory funnel and enough water was added to effect separation of the oil, which was removed. The alkaline liquor was extracted several times with ether and the combined ether and oil was washed repeatedly with water. After drying the ethereal solution with calcium chloride, the solvent was removed and the residue was distilled under reduced pressure. Finally it was refractionated repeatedly under ordinary pressure, collecting as follows: below 190° 3.0; 190 – 203° 1.0; 203 – 216° 3.5; 216 – 222° 4.8; 222 – 240° 8.0; 240 – 250° 7.5; 250 – 260° 6.0 per cent of the total oil. The first two fractions were so small in amount that no identifications could be made. Treatment with bromine gave no crystalline product.

Analysis of the third (I) and fourth (II) fractions gave the following results:

I. 0.2389 gave 0.6915 and 0.2304 H₂O; 0.2245 gave 0.6523 and 0.2205 H₂O. Found: C, 78.9 and 79.1; H, 10.7 and 10.9.

II. 0.1674 gave 0.4429 CO₂ and 0.1638 H₂O; 0.1342 gave 0.3842 CO₂ and 0.1242 H₂O. Found: C, 78.1 and 78.8; H, 10.7 and 10.5.

Required for C₁₀H₁₈O: C, 77.9; H, 11.7.

Since they were relatively small, the two fractions were mixed, after reserving a portion of II. The mixture was gently oxidized with chromic acid solution, using 52 Gm. of potassium dichromate and 78 Gm. of sulphuric acid in 150 cc. of water. After all of the acid solution had been added, the mixture was heated on a steam-bath for twenty minutes and then distilled under diminished pressure. At no time during the oxidation process was an odor of camphor perceptible, so it may be concluded that borneol is not present.

Terpineol.—The acid liquor remaining from the above distillation was shaken out with ether, and the ethereal solution was washed several times with water and dried with calcium chloride. After distilling off the ether, there remained a light yellow oil with a strong acetic odor. Being not very soluble in sodium carbonate solution, it was redissolved in ether and shaken twice with a 5 per cent solution of this salt. After drying the residue and removing the ether, there was left a light yellow oil with a pleasant, coumarin-like odor. This is undoubtedly the ketolactone previously obtained from terpineol by oxidation, but it could not be obtained in a crystalline state, even after standing in a vacuum desiccator for some weeks.

The sodium carbonate solution, from which the above substance was separated, was acidified and shaken out with ether as before, giving a yellow oil which soon became a crystalline paste. After several recrystallizations from anhydrous ether, the melting point was found to be sharply at 175° C. This is evidently terebic acid, known to be formed as a direct oxidation product of terpineol with chromic acid.

The portion of II which had been reserved was shaken with a concentrated solution of hydrogen iodide, producing a heavy, dark brown oil. This was separated from the aqueous layer, shaken several times with sodium sulphite solution to remove free iodine, washed with water, and allowed to stand. A strong sassafras odor developed but the oil did not crystallize. Although no proof can be presented, terpineol is probably present in the oil.

The other three fractions up to 260° C. were comparatively large, constituting nearly one-fourth of the total oil, but no positive identifications could be made; the analyses indicated ketonic or alcoholic character, but attempts to make oximes or other derivatives failed. Each was soluble in 70 per cent alcohol with turbidity and each gave a color reaction with a mixture of acetic and sulphuric acids, similar to that by cadinene.

That portion of the oil boiling above 260° C. was fractionated at 60-mm. pressure and eventually there were obtained three fractions: 165–180° 8.5; 180–195° 4.5; 195–230° 3.0 per cent of the total oil. All of them were blue or green, especially the last which was a beautiful blue, and each was soluble in 70 per cent alcohol. They also gave with concentrated acetic and hydrochloric acids an intense reddish violet color. The blue substance was soluble in syrupy phosphoric

acid with a dark red color and could be recovered by adding water. These reactions are characteristic (7) of azulene, which has been found in many oils.

The analyses of the last six fractions follow:

Boiling point	222-240	240-250	250-260	165-180	180-195	195-230
Color	Yellow	Green	Green	Green	Blue	Deep blue
Odor	Sassafras		Slight			
Wt. sample	0.1346	0.1254	0.1405	0.1809	0.1173	0.1558
CO ₂	0.3836	0.3562	0.3955	0.5055	0.3287	0.4350
H ₂ O	0.1215	0.1151	0.1164	0.1517	0.1066	0.1388
Carbon, %	77.7	77.4	76.8	76.3	73.9	76.1
Hydrogen, %	10.0	10.3	9.2	9.2	10.0	9.5

The Acids.—Since the amount of ester in the original oil was very small, a correspondingly small quantity of saponified acids could be expected. The alkaline solution was evaporated to a low volume, acidified and distilled with steam. As soon as the distillate came over clear, the residue was cooled, again made alkaline and extracted with ether. Evaporation of the solvent gave no residue, showing the absence of lactones. From the aqueous residue, after acidifying, there was only obtained a minute amount of dark brown oil with an unpleasant odor, which indicated that most of the acids were volatile.

The distillate, which had an odor of butyric or valeric acid, was made alkaline and evaporated to a small volume. A portion heated with alcohol and sulphuric acid developed an odor of ethyl butyrate. The remainder was made neutral and precipitated with silver nitrate, resulting in a white salt which contained 57.6 per cent of silver. The acids were evidently butyric (55.4 per cent) and, to a small extent, acetic (64.6 per cent).

SUMMARY.

The leaves of *Juniperus scopulorum* furnish 0.49 per cent of an oil, whose general properties are described. The terpenes, which constitute about 42 per cent, consist of α -pinene, β -pinene and terpinene, with probably some camphene. The oil contains no borneol, but probably about five per cent of terpineol, partly as butyrate or acetate. There are also present some phenol, a relatively large amount of unidentified oxygenated compounds, and a small quantity of azulene.

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"The people must realize that the ultimate control of many diseases, like cancer, influenza, blood poisoning, infantile paralysis, alcoholism, crime and mental disease, depends upon research by a specially trained group, in the laboratories of medical sciences, and this research must be financed by the people, through their representatives and state and federal legislatures. Neither the profession nor the public, to-day, has appreciated the demand, if they wish full protection against disease, for a greater budget to the existing research laboratories in this country."—JOSEPH COLT BLOOGOOD, M.D.