# THE CHEMICAL COMPOSITION OF THE WOOD AND BARK EXTRACTIVES OF JUNIPERUS HORIZONTALIS MOENCH[1]

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ABSTRACT

The wood of creeping juniper was found to contain about 10% acetone-soluble material. Of this, about one quarter was ligroin soluble and, from this portion, thujopsene, α-cedrene, cuparene, cedrol, widdrol, β-sitosterol, esters of fatty acids and β-sitosterol, the lignan savinin, and the recently discovered diterpene communic acid were isolated. Three unidentified hydrocarbons and several unknown alcohols and ketones were also obtained in small amounts. The ether-insoluble portion consisted of intractable polyphenolic material. The composition of the ligroin-soluble extract of the bark of this shrub resembled that of the wood in many respects. Neither the wood nor the bark extractives contained thujaplicins in detectable amounts.

Creeping juniper (*Juniperus horizontalis* Moench; also *Sabina horizontalis* (1)) is a prostrate shrub which is found widely distributed across Canada and occurs frequently in the dry, sandy regions of the Prairie Provinces. A survey of the literature did not reveal any reference to chemical investigations of the components of this plant. This paper deals with some of the components found in the wood and bark of the conifer.

The wood, after removal of the bark, was milled and extracted with acetone, giving 9–10% of soluble material. This extract was then fractionated into various groups of compounds as shown in Fig. 1. When the wood was extracted directly with ligroin, the same yield of ligroin-soluble material was obtained and this was divided into neutral, phenolic, and acidic material in the usual manner. The major portion consisted of neutral components which were fractionated by chromatography on a silicic acid column into five distinct classes of compounds: hydrocarbons, esters, mixed ketones and alcohols, sitosterol, and a lactone, respectively.

*Hydrocarbons*

The hydrocarbon fraction (0.22% of the weight of wood) was analyzed by gas–liquid chromatography (GLC) (2), which showed the presence of one major and five minor sesquiterpene hydrocarbons. The retention times of these components corresponded with six of the seven sesquiterpene hydrocarbons found in commercial cedar wood oil (from *Juniperus virginiana*). Runeberg (3) has identified three of these to be α-cedrene, thujopsene, and cuparene. The first peak recorded from the mixture from creeping juniper had the retention time and infrared spectrum of α-cedrene. The second and major constituent corresponded in retention time to either thujopsene or the isomer of cedrene (4) which is obtained when cedrol is dehydrated by the method of Motl *et al.* (5). This component was not attacked by cold, dilute permanganate solutions, nor did it yield any identifiable product on dehydrogenation with either selenium or palladized charcoal. On oxidation with selenium dioxide, a crystalline aldehyde, m.p. 71–72° C, was obtained. This, together with the GLC and infrared analysis, suggested the major hydrocarbon to be thujopsene. This was confirmed by oxidizing an authentic sample of thujopsene (kindly supplied by Prof. H. Erdtman and T. Norin, Stockholm, Sweden) with selenium dioxide to thujopsenal (6), m.p. 71.5–72° C, which showed no depression in melting point with

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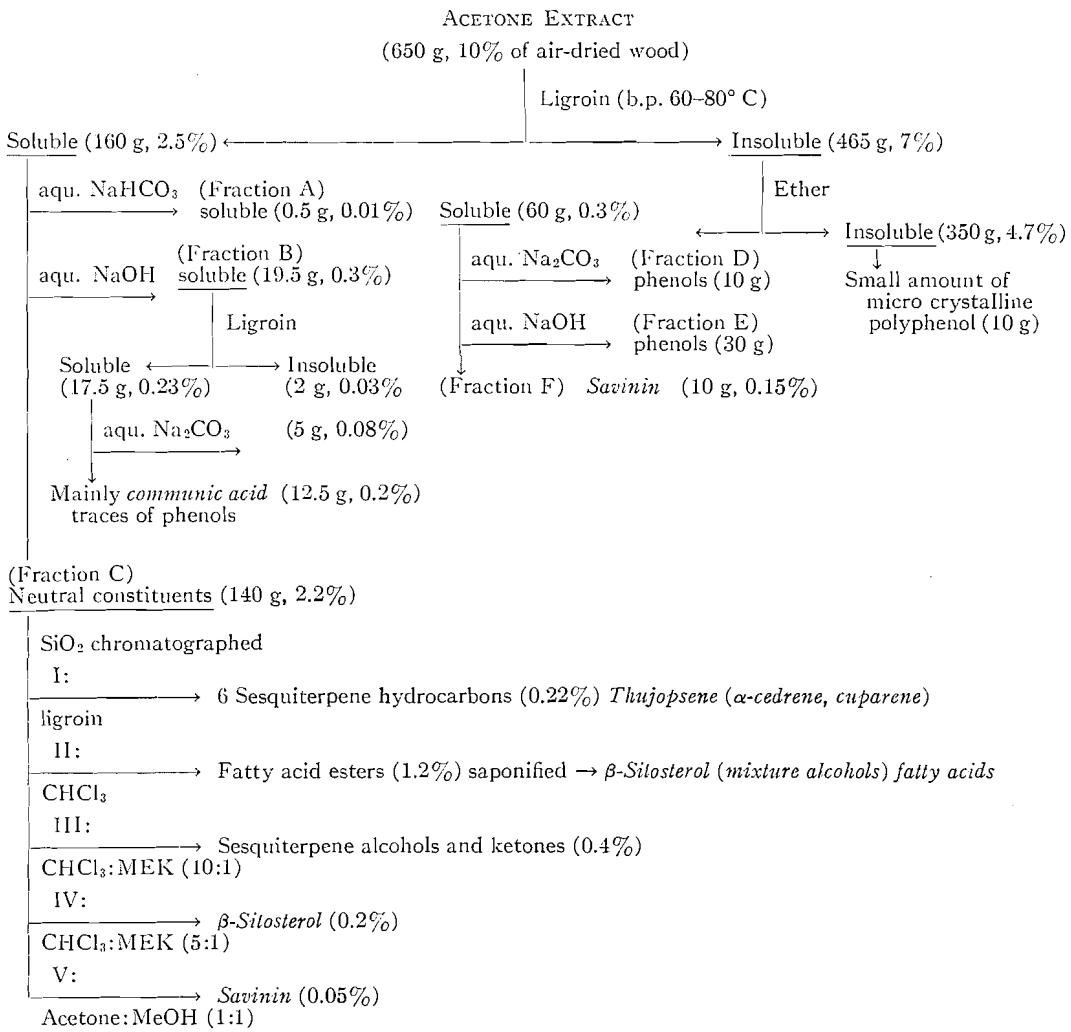


FIG. 1. Fractionation scheme for the wood extractives.

the above aldehyde. It was found that although thujopsene did not react with cold potassium permanganate solution, a liquid diol could be prepared from it with osmium tetroxide. Of the other minor hydrocarbons, none could be obtained pure. However, a concentrate of the second last peak gave an infrared spectrum resembling that of cuparene.

*Esters*

The ester fraction (1.2%) was saponified and from the non-saponifiable portion β-sitosterol and a small amount of a mixture of aliphatic alcohols were obtained. GLC analysis of the latter mixture showed two peaks in the C-12 range, one in the C-15, and one in the C-20 range. The acid portion consisted of a mixture of long-chain fatty acids which was analyzed by GLC (7). Peaks corresponding to palmitic, palmitoleic, stearic, oleic, linoleic, linolenic, and arachidic acid were recorded in the ratio of 6:1:1:13:37:6:2. The high proportion of the unsaturated $C_{18}$ acids is typical for conifer wood (8).

*Ketones and Alcohols*

The third fraction (0.4%) from the silicic acid chromatogram was found to be a mixture of sesquiterpene alcohols and ketones. GLC analysis on the adipate polyester column showed the presence of two components having retention times corresponding to those of cedrol and widdrol, four minor constituents, and a major constituent with a very high retention time. Preparative GLC (20 mg scale) led to the isolation of milligram quantities of the cedrol–widdrol mixture, a mixed ketone–alcohol fraction, and the slow-moving major component. The cedrol–widdrol mixture was resolved on the polyester column and the identity of each alcohol was substantiated by its infrared spectrum. The identity of these two alcohols was further verified by dehydrating the mixture with the pyridine-modified alumina catalyst (4) to a product consisting mainly of $\alpha$-cedrene and thujopsene (GLC). The second fraction had strong hydroxyl and carbonyl absorption bands in the infrared region. Re-chromatography of this fraction on the polyester column gave at least three peaks with strong indications that decomposition took place. The third fraction corresponded to the component having a very high retention time on the polyester column. Its infrared spectrum had absorption peaks at 1665 and 1618 $cm^{-1}$, and a maximum at 235 m$\mu$ with a weak shoulder 285 m$\mu$ was recorded in the ultraviolet region. Attempts to isolate and purify the ketones by means of Girard P reagent or column chromatography failed, mainly because of extensive decomposition of one or more of the components.

*Sitosterol*

Free $\beta$-sitosterol (0.016%) was obtained crystalline from the fourth fraction. No volatile constituents could be detected by GLC in the residue obtained from the mother liquors.

*Lactone*

Of more interest was the last fraction (0.05%) from the chromatogram which was also obtained in crystalline form. The purified, light yellow compound had m.p. 146–147°, $[\alpha]_D^{20}$ −82.3°, and a molecular weight of 362. The ultraviolet spectrum showed maxima at 237, 294, and 334 m$\mu$, whereas the infrared spectrum (KBr disk and $CHCl_3$ solution) had two strong adsorption bands at 1740 and 1640 $cm^{-1}$, indicating the presence of an $\alpha,\beta$-unsaturated lactone. The presence of a lactone ring was confirmed by the solubility of the compound in alcoholic alkali, from which the parent compound was recovered unchanged on acidification. The compound was insoluble in cold ethanol or cold aqueous alkali. Except for the position of the infrared bands discussed above, these data suggested a close resemblance with savinin. The structure of this lignan lactone was elucidated by Hartwell *et al.* (9, 10), who reported infrared absorption peaks at 1751 and 1651 $cm^{-1}$ and also that the compound formed colorless crystals. The mixed melting point of authentic savinin (kindly supplied by Drs. J. L. Hartwell and A. W. Schrecker, Bethesda, U.S.A.) with the yellow compound isolated from creeping juniper was not depressed. The authentic sample also gave absorption bands at 1740 and 1640 $cm^{-1}$ on the infrared spectrophotometer used in these experiments, and agreed in all other respects as well. Thus, there is no doubt that the two compounds are identical.

*Acids and Phenols*

The acidic portion of the ligroin- and ether-soluble extract was found to consist mainly of a diterpene acid (0.25%) $C_{20}H_{30}O_2$, m.p. 121–122° C, $[\alpha]_D^{25}$ +40.3°. On treatment with diazomethane the acid readily formed a methyl ester having m.p. 108–109° C, $[\alpha]_D^{25}$ +50.5°, which could not be saponified even at 125° C. GLC analysis showed the

methyl ester to be a single compound. The infrared spectrum showed the presence of a terminal methylene group (890 $cm^{-1}$) and a trisubstituted double bond (1645 and 795 $cm^{-1}$). Hydrogenation over platinum oxide catalyst resulted in the uptake of hydrogen equivalent to three double bonds. These data suggested that the acid was identical with the sirupy communic acid, isolated recently by Arya *et al.* from the wood of *Juniperus communis* L. (11). This was confirmed by preparing the maleic anhydride adduct of the ester, m.p. 170–171° C, $[\alpha]_D^{25}$ +79.5°. It is to be noted that in the preparation from *Juniperus horizontalis* the acid was obtained readily in the crystalline state.

Small amounts of phenolic materials were also obtained from the acidic portion. Paper chromatography (12) did not reveal the presence of any thujaplicins or related tropolones. This is surprising, since Runeberg and Pilo (13, 14) have found thujaplicins and nootkatin in a large number of juniper species. Thus, the extractives of creeping juniper wood more nearly resemble those of *Chamacyparis thyoides* (15), which are reported to contain no thujaplicins, but α-cedrene, cuparene, thujopsene, cedrol, widdrol, and carvacrol methyl ether. The finding of thujopsene, α-cedrene, cuparene, cedrol, widdrol, and communic acid does show, however, the close biogenetic relationship of the extractive components of creeping juniper with the other juniper species investigated.

The major portion (about 75%) of the acetone-soluble extractives of the wood of creeping juniper consisted of a brown, resinous phenolic material. Various fractionation attempts resulted only in the isolation of a small amount of microcrystalline polyphenolic material. The separation and identification of individual components from this so-called "phlobaphene" fraction will be studied further when more suitable fractionation techniques have been found.

*Bark Extractives*

It was of interest to determine whether the terpenoid compounds found in the wood could also be isolated from the bark of creeping juniper. The bark was milled to a fine powder, extracted with ligroin, and the extract was divided into neutral and acidic constituents. Column chromatography of the neutral portion gave as the first fraction hydrocarbons which appeared from GLC analysis to contain the same components as found in the corresponding fraction of the wood extractives. However, the component having the retention time of α-cedrene was present in about three times the amount of thujopsene. Treatment of the mixture with aqueous permanganate did not result in the formation of α-cedrene diol (16). Oxidation of the mixture with selenium dioxide produced a liquid aldehydic material in high yield which gave, on GLC analysis, only a peak corresponding to thujopsenal. Thus it may be that the unknown major hydrocarbon is an isomer of thujopsene rather than α-cedrene.

The second fraction from the column was composed of esters of β-sitosterol and a mixture of fatty acids. A mixed ketone–alcohol fraction was also isolated but GLC data showed that the individual components differed from those found in the same fraction obtained from the wood. In addition, an ester fraction was isolated which was composed of alcohols having terpenoid character and a mixture of $C_{16}$, $C_{18}$, and $C_{20}$ acids in which the latter predominated. The alcoholic material could not be fractionated by column chromatography and characterization will be attempted by preparative GLC, once this technique has been perfected.

Savinin was isolated from the bark extract in small, though better, yield than was obtained from the wood. The major acidic component was again communic acid. Small amounts of untractable phenolic material were also obtained. Thus, the composition of the ligroin extract of the bark resembled that of the wood in many respects.

## EXPERIMENTAL

Melting points were determined on a Leitz hot stage microscope. The identity of known compounds was confirmed by mixed melting point and by comparing the infrared spectra with those of authentic samples unless otherwise indicated. The infrared spectra were recorded on a Perkin–Elmer Model 21 double-beam recording spectrophotometer using the KBr-disk method, the film technique, or solutions in carbon tetrachloride. GLC chromatograms were obtained with a modified Beckman GC-2 chromatograph, using either 6 ft$\times\frac{1}{4}$ in. O.D. coiled copper columns packed with adipate (2) or succinate (7) polyester and "Chromosorb W" (80 to 100 mesh) in the ratio of 1:6, an 18 in.$\times\frac{1}{4}$ in. O.D. column containing washed silicone grease (2) on Celite (100 to 120 mesh) in the ratio of 1:6, or a 3 ft$\times\frac{1}{4}$ in. O.D. stainless steel column containing SE-30 silicone rubber (Dow Corning) on "Chromosorb W" (40 to 60 mesh) in the ratio of 1:3. The commercial cedar wood oil (Fritzsche Bros. of Canada Ltd., Toronto) used for comparison was obtained from the wood of *Juniperus virginiana* L.

### *Extraction of the Wood*

The plants were collected near Saskatoon in the early fall of 1960 and in the spring of 1961. The leaves and bark were separated mechanically from the wood and the wood was milled to a fine powder by means of a hammer and a Wiley mill. The air-dried, milled wood (6.5 kg) was extracted continuously with acetone in a Soxhlet extractor for 24 hours and the extract was reduced to a brown, viscous sirup by distilling off most of the acetone. A small aliquot was evaporated to dryness to determine the yield of acetone-soluble material. The residue was poured into excess ligroin (b.p. 60–80° C) (4 liters) and the clear ligroin solution was decanted from the brown, resinous, insoluble material. The latter was washed twice with ligroin and the washings were added to the ligroin extract. The combined ligroin extract (6 liters) was concentrated to 2 liters by distillation. The yield of acetone-soluble material (650 g) was about 10% of the weight of air-dried wood, and that of the ligroin-soluble portion was 2.5%. The ligroin-soluble material could be obtained in about the same yield by extracting the milled wood directly with ligroin in a Soxhlet extractor.

### *Preliminary Fractionation*

The concentrated ligroin extract (160 g in 2 liters) was extracted with a saturated solution of sodium bicarbonate and washed with water. The aqueous extracts were combined, acidified with cold dilute hydrochloric acid, and extracted with ether. The ethereal solution was dried over anhydrous sodium sulphate and after filtration evaporated to dryness to yield 0.5 g of a brown sirupy material (fraction A). The ligroin solution was then further extracted with two portions (100 ml) of aqueous sodium hydroxide solution (5%). The ligroin layer was again washed with water and the aqueous solutions pooled. The latter was acidified with cold, dilute hydrochloric acid, extracted with ether, and worked up as above to yield 19.5 g of weakly acidic material (fraction B). The residual ligroin solution was dried over anhydrous sodium sulphate and, after filtration, evaporated to dryness to yield 140 g of neutral oil (fraction C). When a small aliquot of the neutral material was distilled on a Widmer column, decomposition was encountered. About 20% of the material distilled between 110° and 140° C at 3 mm pressure. It was observed that chromatography on a silicic acid column, followed by vacuum distillation, gave a better separation of the volatile components.

*Silicic Acid Column Chromatography*

The neutral oil (10 g) in chloroform was chromatographed on a 2.0-cm diameter column of silicic acid (100 g) slurried in ligroin (b.p. 40–60° C). The column was eluted successively with ligroin (b.p. 40–60° C), chloroform, chloroform–2-butanone (10:1 and 5:1 v/v), and acetone–methanol (1:1 v/v), the solvent being changed when the last 50 ml of eluate gave no residue on evaporation. Five distinct fractions were collected in 1.2, 4.8, 1.6, 0.6, and 0.2 g amounts respectively. The infrared spectra showed the first to contain hydrocarbons, the second esters, the third a mixture of ketones and alcohols, the fourth alcohols, and the last a lactone.

*Fraction I: Thujopsene and other Sesquiterpene Hydrocarbons*

The material was analyzed by GLC on the adipate polyester column at 180° C. Six peaks were recorded in the sesquiterpene hydrocarbon range in 5, 72, 3, 6, 5, and 8% amounts respectively. The relative retention times (RRT; $\alpha$-cedrene = 1.00) were 1.02, 1.15, 1.57, 1.74, 1.97, and 2.28 respectively. When commercial cedar wood oil, obtained from *Juniperus virginiana*, was injected, seven peaks were recorded, six of which corresponded in retention times to the above peaks. Fractional distillation gave a middle cut, b.p. 110–120° C (air bath) at 3 mm pressure, which contained about 88% of the major component. Found: C, 87.41%; H, 11.60%; $n_D^{26}$ 1.5049; $[\alpha]_D^{26}$ −44.4 ($CHCl_3$). Calculated for $C_{15}H_{24}$: C, 88.16%; H, 11.84%. The crude hydrocarbon was unaffected by cold neutral or alkaline solutions of potassium permanganate. Selenium dehydrogenation (at 280 to 320° C) did not yield any identifiable product, nor was a crystalline picrate obtained. When the crude hydrocarbon was hydrogenated, using either palladized charcoal or platinum oxide as catalyst, the main hydrocarbon was recovered unchanged (GLC).

The crude hydrocarbon (300 mg), selenium dioxide (200 mg), and ethanol (30 ml) were heated under reflux for 4 hours. The ethanol was removed by evaporation under reduced pressure and ether was added to the residue. The ethereal solution was decanted from the residual selenium metal and the ether-soluble material (which contained appreciable amounts of selenium) was distilled *in vacuo* to give a pale yellow liquid, b.p. 110° C (air bath) at 3 mm pressure, which solidified partially on standing for some time. The distillate was taken up in ligroin (1 ml) and left at 2–5° C for several days, when the product crystallized in colorless, rectangular rods, m.p. 71–72° C after two recrystallizations. Found: C, 82.54%; H, 10.25%. Calculated for $C_{15}H_{22}O$: C, 82.51%; H, 10.16%. When an authentic sample of thujopsene was oxidized in the same manner thujopsenal of m.p. 71.5–72° C was obtained.

A few milligrams of the first peak were obtained almost pure by preparative GLC (20 mg scale) (2). The material had an infrared spectrum similar to that of $\alpha$-cedrene. The presence of cuparene was inferred from the infrared spectrum of a concentrate of the second last peak, which was similar to that of this aromatic hydrocarbon.

*Thujopsene diol.*—Thujopsene (85% pure) (250 mg) and osmium tetroxide (250 mg) were dissolved in anhydrous ether (30 ml) and pyridine (1 ml). A brown, fluffy precipitate formed. The mixture was left at room temperature for 24 hours. It was then centrifuged, the solvent removed by decantation, and the solid residue was washed with ether. To the residue was added sodium bisulphite (1.5 g) in water (10 ml) and ethanol (30 ml), and the mixture was heated on a steam bath for 4 hours. The cooled solution was filtered and the alcohol was removed by evaporation *in vacuo*. The residual aqueous solution was extracted

repeatedly with ether and the ethereal extract was dried over anhydrous sodium sulphate. The oily residue obtained on evaporation of the ether contained no starting material (GLC) and showed a strong hydroxyl absorption band in the infrared region. The diol crystallized from ether–ligroin (1:1 v/v) at 2–5° C but liquefied immediately upon filtration, even in the cold.

*Fraction II: Esters of β-Sitosterol, Aliphatic Alcohols, and Long-Chain Fatty Acids*

This fraction was a viscous oil with a fatty odor. The infrared spectrum showed, as the only significant band, an ester carbonyl absorption (1740 $cm^{-1}$). The material was, therefore, saponified with alcoholic potassium hydroxide (steam bath, 2 hours) and worked up into the neutral and acidic components in the usual manner. Silicic acid chromatography of the neutral portion gave two distinct fractions. The first (10%) was analyzed by GLC on the silicone column at 160 and 200° C and was found to consist of four alcohols, two in the C-12, one in the C-15, and one in the C-20 range. The second fraction (90%) crystallized from acetone and was found to be β-sitosterol, m.p. 139–140° C, acetate m.p. 132–133° C. An aliquot of the acid portion was methylated with diazomethane and the mixture of methyl esters was analyzed by GLC on the succinate polyester column at 205° C. Peaks corresponding in retention time to palmitic (9.1%), palmitoleic (1.5%), stearic (1.5%), oleic (19.1%), linoleic (55.5%), linolenic (9.1%), and arachidic (3.03%) acids were recorded.

*Fraction III: Mixed Alcohols and Ketones*

Infrared analysis indicated that this fraction was composed of alcohols (3440 $cm^{-1}$) and ketones (1667 $cm^{-1}$). Found: C, 81.93%; H, 10.55%. Calculated for $C_{15}H_{24}O$: C, 81.76%; H, 10.98%. The mixture was analyzed by GLC, using the adipate polyester column at 180° C. Seven peaks were recorded in the ratio of 9:20:1:2:1:2:5:65. The respective RRT (cedrol = 1.00) were 1.02, 1.16, 1.44, 1.62, 1.94, 2.50, and 3.80. Some decomposition appeared to take place. Preparative runs of 20-mg aliquots on the SE-30 column yielded 5 to 10 mg each of three major components. These were re-chromatographed on the adipate polyester column, when the first fraction was resolved into two peaks corresponding in retention time to cedrol and widdrol. Sufficient material could be collected to record the infrared spectra of each component and these corresponded with those of cedrol and widdrol. The mixture (10 mg) was heated with alumina (30 mg) containing pyridine (1%) in a 5-mm glass tube (4) at 230° C for 1 hour. After it was cooled, the product was extracted with ether, and the ethereal solution was filtered and then evaporated to dryness. When the residue was chromatographed on the adipate polyester column, two major peaks, corresponding in retention time to α-cedrene and thujopsene, were recorded. The second fraction from the preparative run was found to decompose extensively on re-chromatographing on the polyester column; one major (RRT 1.62) and two minor peaks (RRT 1.44 and 1.94) were recorded but all were ill-defined. The infrared spectrum of the collected fraction showed strong absorption bands at 3360, 2900, 1675, (1650), 1460, and 1377 $cm^{-1}$ and medium ones at 885, 875, 815, and 705 $cm^{-1}$. The ultraviolet spectrum showed a broad maximum at 245 mμ. The third fraction was composed of the component having RRT 3.80 on the polyester column. It had a weak infrared spectrum with absorption bands at 2960, 2925, 1665, 1618, 1455, 1435, 1387–1372, 1355, 1295–1285, 1200, and 885–870 $cm^{-1}$. Its ultraviolet spectrum showed a maximum at 235 mμ with a weak shoulder at 282 mμ.

*Fraction IV: β-Sitosterol*

This fraction crystallized on reducing the volume of solvent. Recrystallization from methanol gave white plates of β-sitosterol, m.p. 138–139° C, acetate m.p. 132–133° C.

*Fraction V: Savinin*

The yellow sirup obtained on evaporation of the solvent was taken up in acetonitrile and allowed to stand at 2–5° C for several days. A crystalline substance, m.p. 135–140° C, was obtained which was recrystallized twice from ethanol; m.p. 146–147° C, $[\alpha]_D^{25}$ −82.3° (*c*, 0.6, $CHCl_3$). Found: C, 68.50%; H, 4.75%; molecular weight (Rast), 362. Calculated for $C_{20}H_{16}O_6$: C, 68.18%; H, 4.58%; molecular weight, 352.

The compound is sparingly soluble in cold alcohol, insoluble in cold aqueous sodium hydroxide solution, and was recovered unchanged on acidifying an alcoholic solution containing sodium hydroxide. The ultraviolet spectrum showed maxima at 334, 294, and 237 mμ and minima at 306 and 266 mμ and alkaline solutions fluoresced strongly. The infrared spectrum had strong carbonyl absorption bands at 1740 and 1640 $cm^{-1}$. The position of these bands did not change on standardizing the infrared spectrophotometer against indene or polystyrene film. The authentic sample of savinin, m.p. 146.2–147.3° C, $[\alpha]_D^{22}$ −88.2°, also gave absorption bands at 1740 and 1640 $cm^{-1}$ with the standardized instrument.

*Acidic Components*

Both the fractions obtained from the aqueous bicarbonate (fraction A) and sodium hydroxide solutions (fraction B) were investigated for the presence of tropolones. The paper chromatographic procedure of Wachtmeister and Wickberg (12) did not reveal any thujaplicins or nootkatin. A number of weak spots corresponding to phenols (ferric chloride color reaction) were obtained with butanol – acetic acid – water (4:1:2) as solvent.

The acid fraction B (see above) was triturated twice with ligroin (250 ml each) and the solution was decanted from the insoluble residue. The solution was extracted with aqueous sodium carbonate solution. The alkaline solution was separated, acidified with dilute hydrochloric acid, and extracted with ether. The ethereal solution was dried, filtered, and evaporated. The residue (5.0 g) was chromatographed on a column of silicic acid when three distinct fractions were collected. Paper chromatography again showed the absence of tropolones.

The carbonate-extracted ligroin solution was dried over anhydrous sodium sulphate and, after filtration, evaporated to dryness. The residue (17.5 g) was chromatographed on silicic acid. The major portion was eluted with ligroin (b.p. 40–60° C) and the residue obtained on evaporating the solvent (12.5 g) crystallized from acetone–ligroin in rectangular plates, m.p. 121–122° C; $[\alpha]_D^{25}$ +40.3° (*c*, 0.6, $CHCl_3$). Found: C, 79.31%; H, 9.97%; COOH, 15.23%. Calculated for $C_{20}H_{30}O_2$: C, 79.42%; H, 10.00%; COOH, 15.0%. The acid formed a cyclohexylamine salt from which the free acid could be regenerated. Treatment with diazomethane gave the crude methyl ester, m.p. 101–102° C. On recrystallization twice from methanol, the melting point was raised to 108–109° C. Found: C, 79.64%; H, 10.08%; $[\alpha]_D^{26}$ +50.5° (*c*, 2%, $CHCl_3$). Calculated for $C_{21}H_{32}O_2$: C, 79.70%; H, 10.19%. The methyl ester could not be hydrolyzed, even on prolonged treatment with potassium hydroxide in 2-methoxyethanol at 125° C. Microhydrogenation in methanol – acetic acid (4:1) with platinum oxide gave a hydrogen uptake of 3 mole equivalent. The maleic anhydride adduct of the methyl ester was prepared in the usual manner; m.p. 171° C, $[\alpha]_D$ +79.5°. Arya *et al.* (11) report for the methyl ester m.p. 105–106° C, $[\alpha]_D$ +47.0° and for the maleic anhydride adduct, m.p. 169–171° C, $[\alpha]_D$ +85°. Hydrogenation of the methyl ester in methanol over platinum oxide gave the hexahydro derivative. Found: C, 78.39%; H, 11.50%; $[\alpha]_D^{25}$ +50.75° (*c*, 1.2, $CHCl_3$). Calculated for $C_{21}H_{36}O_2$: C, 78.26%; H, 11.8%. This methyl ester could also not be saponified.

*Ether-soluble Material*

The resinous residue (465 g) after ligroin extraction was extracted twice with boiling ether (1 liter) and the ether was decanted from the viscous residue. The extract was filtered and concentrated (500 ml). A small aliquot was evaporated to dryness and the total yield was calculated to be 60 g. The ethereal solution was then successively extracted with 5% aqueous solution of sodium carbonate (fraction D) and sodium hydroxide (fraction E), washed with water, dried over sodium sulphate, and the solvent was distilled off (fraction F).

*Fractions D and E (40.0 g)*

On acidification a bright red oil was obtained which was taken up in ether. Attempts to crystallize the material failed. With alcoholic ferric chloride solution a bright green color was obtained. Paper chromatography (12) did not reveal the presence of any thujaplicins or nootkatin.

*Fraction F (10.0 g)*

On long standing the concentrated solution in ethanol deposited yellow prisms (0.2 g), m.p. 142–144° C. The infrared spectrum was similar to that of savinin in all respects and the mixed melting point was undepressed.

*Acetone-soluble Residue (350 g)*

The residue was taken up in acetone (1.5 liter) and allowed to stand in the cold for several weeks when a small quantity (10 g) of microcrystalline brown material was deposited. The filtered and dried material gave a positive ferric chloride reaction. Its infrared spectrum confirmed the phenolic character.

*Extraction and Analysis of the Bark*

The finely powdered bark (2.1 kg) was extracted continuously with ligroin (b.p. 60–80° C) in a Soxhlet extractor for 24 hours. The soluble material (71 g) was divided into neutral (41 g) and acidic (26 g) fraction as above.

*Neutral Components*

Silicic acid chromatography (see above) gave again five fractions. The first fraction (3.0 g) was composed of sesquiterpene hydrocarbons and a small amount of the esters of fraction 2. The sesquiterpenes were purified by distillation *in vacuo*. GLC analysis gave seven peaks with RRT, 0.85, 1.00, 1.19, 1.62, 1.76, 2.29, and 2.50 in 1, 63, 21, 8, 2, 1, and 3% amounts respectively. The two major peaks corresponded in retention time to $\alpha$-cedrene and thujopsene. However, permanganate oxidation did not give the expected $\alpha$-cedrene diol. Selenium dioxide oxidation gave a liquid aldehyde which showed a single peak with retention time equal to thujopsenal on GLC analysis.

The second fraction (20 g) was composed of fatty acid esters and was again found to yield $\beta$-sitosterol, m.p. 136–137° C, and a mixture of fatty acids on saponification. Fraction 3 (5.0 g) differed from fraction III of the neutrals of the wood. The infrared spectrum indicated esters of long-chain fatty acid. Saponification with alcoholic sodium hydroxide gave a sesquiterpene alcohol fraction (infrared, GLC) and a fatty acid mixture in which two $C_{20}$ acids predominated. GLC analysis of the derived methyl esters showed the $C_{16}$, $C_{18}$, and $C_{20}$ acids to be present in the ratio of 1:2.5:3. From the mixture of acids a crystalline product, m.p. 65–70° C, was obtained in small yield. GLC analysis of this material showed it to be composed mainly of arachidic acid, with palmitic and stearic acid as contaminants. The fourth fraction (3.0 g) was divided into a volatile (b.p. 140–160° C at 3 mm) and a non-volatile component. The latter (0.6 g) crystallized from

ligroin in colorless needles, m.p. 136–137° C undepressed in admixture with β-sitosterol, m.p. 137–138° C. The infrared of the volatile material showed bands in the hydroxyl and carbonyl region. GLC analysis showed a similar mixture of ketones and alcohols as was obtained from fraction III of the wood neutrals, but the retention times of all components differed from those of the latter. The last fraction (5.0 g), when taken up in a little ethanol, again deposited small amounts of yellow needles of savinin, m.p. 145–146° C after recrystallization.

*Acidic Components*

The acidic material was chromatographed on silicic acid, using the same solvent as described above. The major portion (15 g) was found to be communic acid (infrared, mixed melting point). The crude acid was converted to the methyl ester with diazomethane and analyzed by GLC on the silicic acid column at 230° C. Only a single peak having the retention time of methyl communate was recorded. Three small viscous fractions were also obtained from the chromatogram, none of which crystallized. The last two gave a positive color reaction for phenols with ferric chloride solution.

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REFERENCES

1. W. P. Fraser and R. C. Russel. An annotated list of the plants of Saskatchewan. Revised edition. University of Saskatchewan, Saskatoon, Sask. 1954. p. 3.
2. E. von Rudloff. Can. J. Chem. **38**, 631 (1960).
3. J. Runeberg. Acta Chem. Scand. **14**, 1288 (1960).
4. E. von Rudloff. Can. J. Chem. **39**, 1860 (1961).
5. O. Motl, V. Herout, and F. Sorm. Collection Czechoslov. Chem. Communs. **23**, 1293 (1958).
6. H. Erdtman and T. Norin. Acta Chem. Scand. **13**, 1124 (1959).
7. B. M. Craig and N. L. Murty. J. Am. Oil Chemists' Soc. **36**, 549 (1959).
8. M. A. Buchanan, R. V. Sinnett, and J. A. Jappe. Tappi, **42**, 578 (1959).
9. J. L. Hartwell, J. M. Johnson, D. B. Fitzgerald, and M. Belkin. J. Am. Chem. Soc. **75**, 235 (1953).
10. A. W. Schrecker and J. L. Hartwell. J. Am. Chem. Soc. **76**, 4896 (1954).
11. V. P. Arya, C. Enzell, H. Erdtman, and T. Kubota. Acta Chem. Scand. **15**, 225 (1961).
12. C. A. Wachtmeister and B. Wickberg. Acta Chem. Scand. **12**, 1335 (1958).
13. J. Runeberg. Acta Chem. Scand. **14**, 1985, 1991, 1995 (1960).
14. C. Pilo and J. Runeberg. Acta Chem. Scand. **14**, 353 (1960).
15. C. Enzell. Acta Chem. Scand. **14**, 81 (1961).
16. E. Guenther. *In* The essential oils. Vol. II. D. van Nostrand, Inc., New York. 1949. p. 116.