SOME EXTRACTIVES FROM

POPULUS BALSAMIFERA L. AND

OPUNTIA FRAGILIS Nutt.

A Thesis

Submitted to the

Faculty of Graduate Studies

in Partial Fulfilment of the

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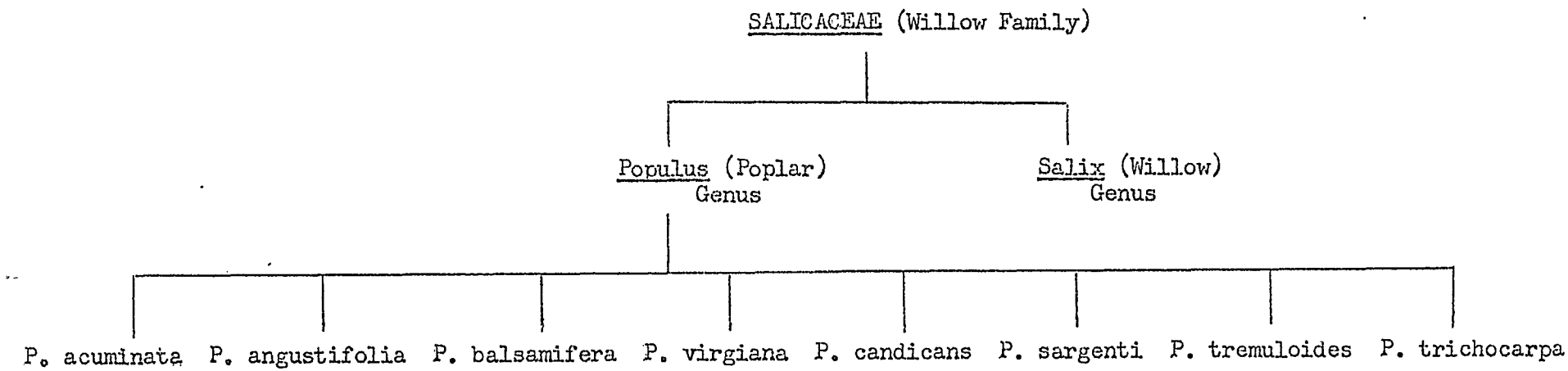
I. INTRODUCTION

The purpose of this thesis is to give an account of a systematic investigation carried out on the extractives of Populus balsamifera and Opuntia fragilis.

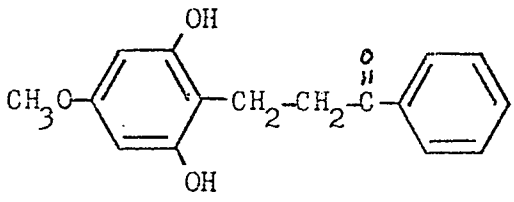
Salicaceae (the Willow family) consist of the Populus (Poplar) genus and the Salix (Willow) genus. Eight species of the Populus genus are known in North America (1) (Schematic I). Of these eight species, only P. balsamifera, P. tremuloides, and P. trichocarpa have drawn the attention of research workers. However P. tremula (European Aspen) and P. grandidentata found elsewhere have been studied quite extensively.

The buds (Balm of Gilead) of P. balsamifera (or P. tacamahaca, otherwise known as Balsam poplar) have been studied in reasonable detail, whereas considerably less work has been reported on the bark and heartwood; virtually nothing has been done on the leaves. A study by Farwell (2) showed that commercial poplar buds are collected from P. balsamifera Linn, and P. tacamahaca Mill. Goris and Canal (3), working with the fresh buds of P. balsamifera, isolated 2',6'-dihydroxy-4'-methoxy-β-phenylpropiophenone (I) from the oil which was identical to the synthetic material they later synthetized (4). Further studies on P. balsamifera buds by Goris and Canal (5) established the presence of asparagine, saccharose, salicoside, cinnamic, propionic, butyric, p-hydroxybenzoic, 3,4-dihydroxycinnamic and 2,3-dihydroxybenzoic acids; a sesquiterpene alcohol, the cinnamyl and phenylethyl esters of cinnamic and lignoceric acids, acetophenone, the hydrocarbons $C_{25}H_{52}$, $C_{27}H_{56}$, and $C_{29}H_{60}$, as well as 2',6'-dihydroxy-4'-methoxy-β-phenylpropiophenone which they reported earlier. Sorm, Urany, and Herout (6) obtained an oil from the steam distillation of P. balsamifera

SCHEMATIC I


SALICACEAE (Willow Family)
Populus (Poplar) Genus
Salix (Willow) Genus
P. acuminata
P. angustifolia
P. balsamifera
P. virgiana
P. candicans
P. sargenti
P. tremuloides
P. trichocarpa

buds from which they characterized cineole (II), an alcohol $C_{10}H_{18}O$, d-cadinene (III), α-curcumene (IV), farnesene (V), bisabolene (VI), and bisabolol (VII). Working with a supposedly neutral portion of the ether extractives of a commercial spent sulfite liquor from the pulping of mixed aspenwood (P. tremuloides, P. grandidentata and P. tacamahaca) Pearl and McCoy (7) found esters of long-chain fatty alcohols, sterols, and glycerol with saturated long chain fatty acids and phenolic acids. After hydrolysis, long-chain saturated fatty alcohols C_{26}, C_{27}, and C_{28} were found. Reversed phase chromatography of the acids indicated the presence of myristic (VIII), palmitic (IX), stearic (X), arachidic (XI), behenic (XII) and lignoceric (XIII) saturated acids, along with the unsaturated acids oleic (XIV), linoleic (XV), linolenic (XVI), and arachidonic (XVII) acids. Paper chromatographic investigations also indicated the presence of vanillin (XVIII), ferulic (XIX), p-coumaric (XX) and p-hydroxybenzoic (XXI) acids. In interpreting these results one must remember that these compounds were obtained from a mixture of species (P. tremuloides, P. grandidentata, P. tacamahaca) so there is no way of knowing from which one specific species the compounds were isolated. Pearl and coworkers (8, 9) isolated and characterized syringaldehyde (XXII), vanillin, p-hydroxybenzaldehyde (XXIII), acetovanillone (XXIV), vanillic (XXV), syringic (XXVI), ferulic, p-hydroxybenzoic and p-coumaric acids from the hydrolysis of P. tacamahaca heartwood and bark. Further work by Pearl and associates (10, 11) resulted in the isolation of salicin (XXVII), trichocarpin (XXVIII), salireposide (XXIX), salicyl alcohol (XXX), gentisyl alcohol (XXXI), and p-coumaric acid from the hot water extractives of green P. balsamifera bark. The presence of other glucosides of salicyl and gentisyl alcohols and gentisic acid was demonstrated by thin layer chromatography.



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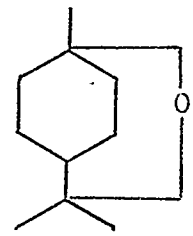

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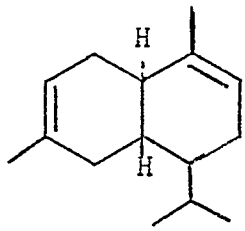

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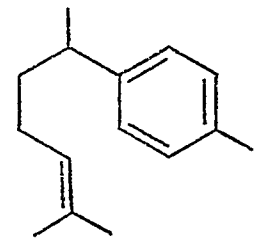

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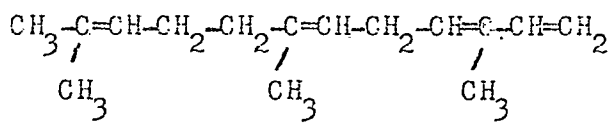

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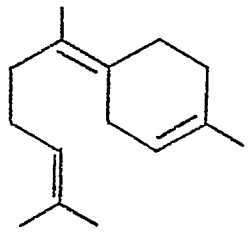

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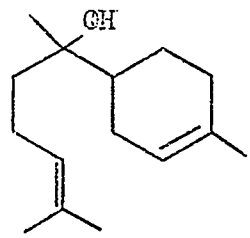


VII

$CH_3(CH_2)_nCOOH$

VIII n = 12

IX n = 14

X n = 16

XI n = 18

XII n = 20

XIII n = 22

$CH_3(CH_2)_7CH{=}CH(CH_2)_7CO_2H$

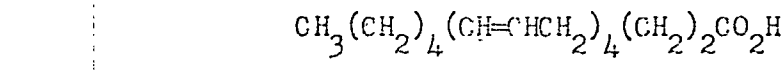
XIV

$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7CO_2H$

XV

$CH_3CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_7CO_2H$

XVI



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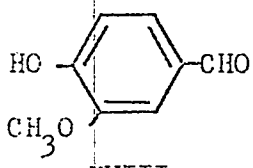

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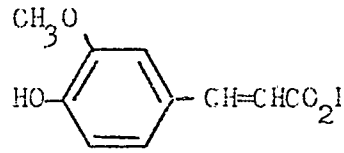

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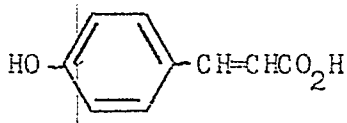
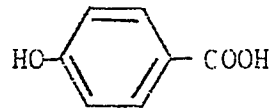
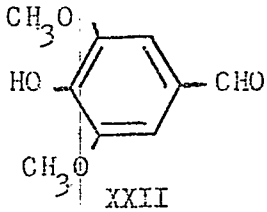


XX



XXI



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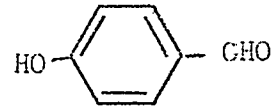

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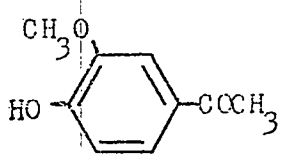

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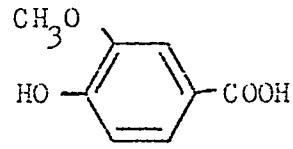

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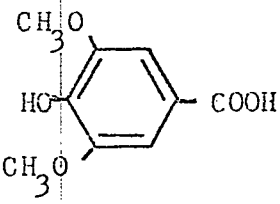

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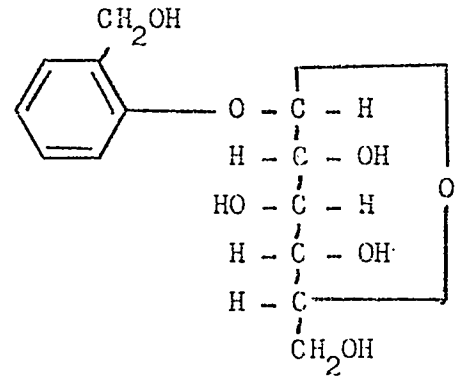

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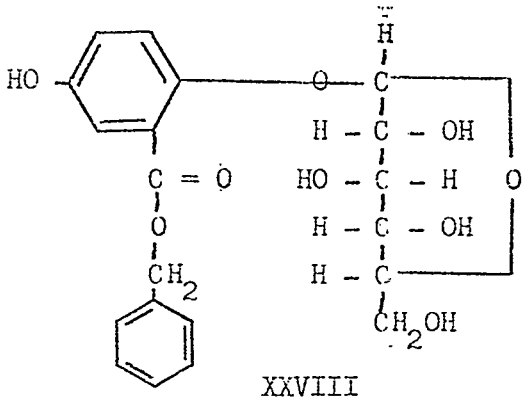
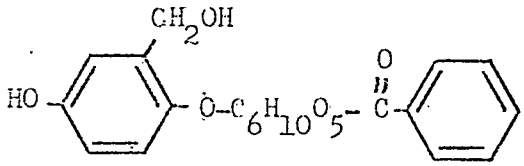


XXVIII



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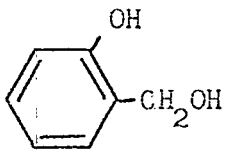
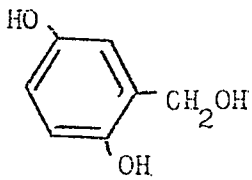


XXX



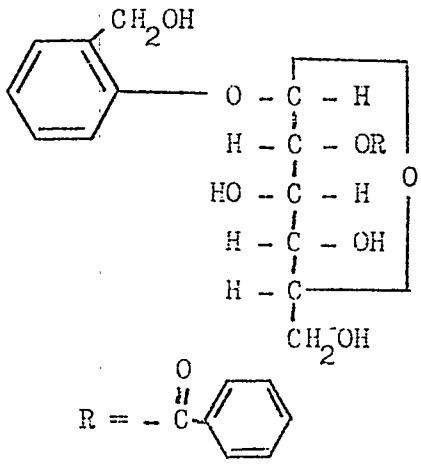
XXXI

The most extensively studied species of the Populus genus is P. tremuloides (Aspen Poplar or Quaking Aspen) and its European counterpart P. tremula. Bridel (12) noted the presence of salicin in P. tremula early in 1920. Much later Perilä (13) identified some of the saturated fatty acids in aspenwood which included the homologous series from C_1 to C_{10} and the even numbered acids to C_{26}. Studying Aspen bark, Hossfeld and Hunter (14) isolated a crystalline hydrocarbon m.p. 57 - 57.5°, an unsaturated β-hydroxy-sterol m.p. 173 - 175°, and identified lignoceric and linolenic acids, β-sitosterol, ceryl alcohol and glycerol after saponification of the

petroleum ether extract. Pearl and Darling (15, 16) isolated and characterized tremuloidin (XXXII), a new glucoside, and salireposide in P. tremuloides bark. Vanillin, syringaldehyde, p-hydroxybenzaldehyde, acetovanillone, vanillic, syringic, p-hydroxybenzoic, p-coumaric and ferulic acids were obtained from the alkaline hydrolysate of P. tremuloides heartwood and bark (8, 9). Faber (17) found that the phloem and bark of Aspen poplar contained p-coumaric, p-hydroxybenzoic, vanillic, benzoic, and possibly ferulic acids. Salicin, populin (XXXIII), tremuloidin and salireposide were tentatively identified. Later Pearl and Beyer (18) detected syringaldehyde in the sapwood and p-hydroxybenzoic acid in the rootwood of P. tremuloides. Using the neutral materials from the benzene extractives of P. tremuloides wood Pearl and Harrocks (19) divided them into methanol-soluble and methanol-insoluble fractions; both fractions were saponified. The saponification products were fractionated to yield all members of the saturated acid series from C_{12} to C_{28} including the odd numbered acids (with the single exception of the C_{27} acid), linoleic and oleic acids, C_{24}, C_{26} and C_{27} saturated fatty alcohols and glycerol. In addition they isolated an alcohol $C_{32}H_{54}O$, and a steroidal compound $C_{32}H_{56}O$, whose respective structures have not been proved. Pearl, Darling, DeHass, Loving, Scott, Turley and Werth (10) detected the presence of salicin, tremuloidin, salireposide, salicyl alcohol, gentisyl alcohol and p-coumaric acid in the hot water extractives of P. tremuloides bark. The presence of sucrose, glucose, fructose, mannose, galactose and two unidentified oligosaccharides was shown tentatively. Populin was isolated from P. tremuloides leaves (20).

Abramovitch, Micetich, and Smith (21), and Abramovitch and Micetich (22, 23) identified all members of the saturated acid series from C_{16} to C_{28}

(except C_{17}, C_{19} and C_{26} acids), a number of normal aliphatic hydrocarbons (C_{14} - C_{19}, and odd chain lengths to C_{29}), three normal aliphatic alcohols (C_{24}, C_{26} and C_{28}), a crystalline ketone identified as stigmasta -3,5-diene-7-one (tremulone) (XXXIV), the unsaturated alcohols α- (XXXV) and β- (XXXVI) amyrin, butyrospermol (XXXVII), lupeol (XXXVIII), 24-methylene-cycloartanyl alcohol (XXXIX), and β-sitosterol (XL) as well as β-sitosterol glucoside from the P. tremuloides heartwood. Further studies on P. tremuloides heartwood by Abramovitch and Koleoso (31) using the steam-volatile fraction of the benzene extract resulted in the isolation and characterization of benzyl alcohol, phenol, β-phenylethanol, p-ethylphenol, n-hexyl alcohol, n-heptyl alcohol and methyl benzoate. Two hydroxy-α, β-unsaturated aldehydes were also isolated from this fraction but could not be characterized. Pearl and Darling (24, 25) have found that mild alkaline treatment or hot basic lead acetate solution used in the isolation of glucosides from P. tremuloides and P. grandidentata bark and leaves causes migration of the benzoyl group from the 2-position in tremuloidin to the 6-position in populin whereas if the extraction was done in the absence of alkali or with a minimum of cold basic lead acetate only tremuloidin was obtained. This observation means that any populin isolated using alkali or lead acetate treatment may be an artifact with tremuloidin being the natural material in the leaves and barks.



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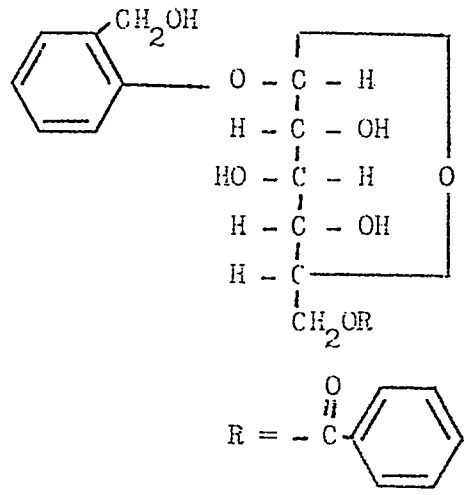

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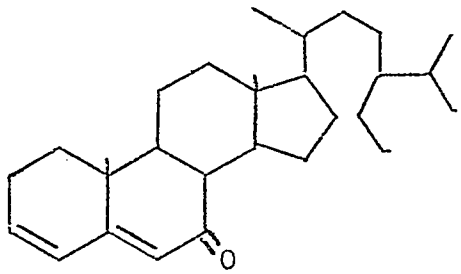

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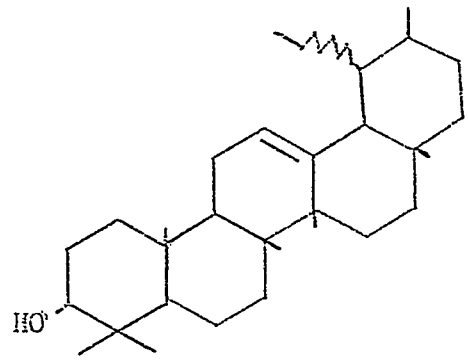

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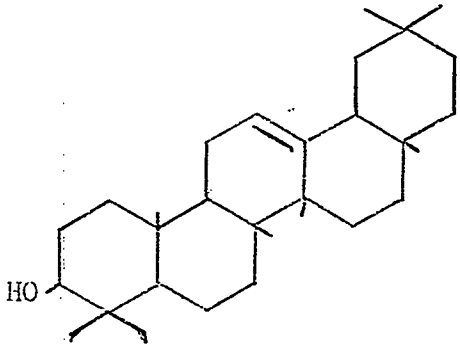

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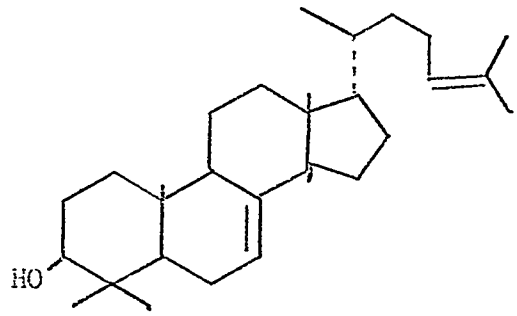
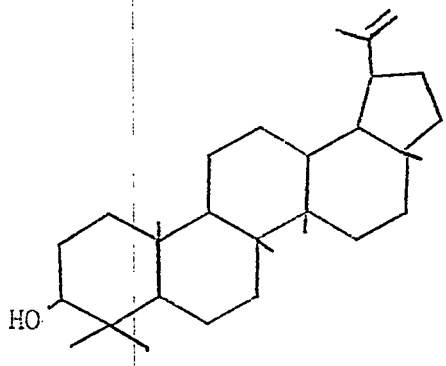
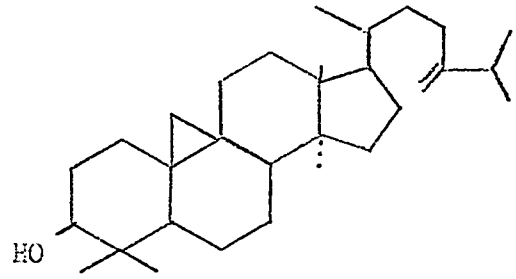


XXXVII



XXXVIII



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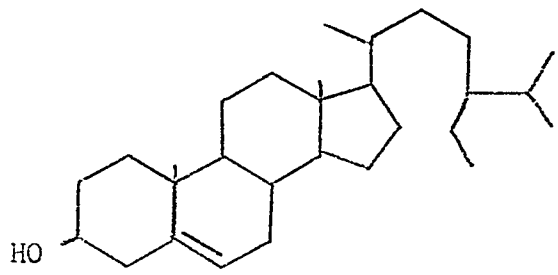
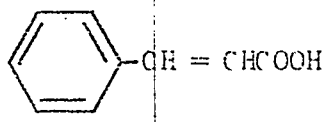


XL

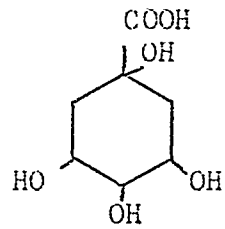
A systematic phytochemical study of P. trichocarpa was not undertaken until the last decade. An earlier investigation by Torr and Gray (26) revealed the presence of cinnamic (XLI), and butyric acids, a paraffin $C_{25}H_{52}$, benzyl salicylate and benzyl benzoate in the buds. Vanillin, syringaldehyde, p-hydroxybenzaldehyde, acetovanillone, acetosyringone, vanillic, syringic, p-hydroxybenzoic, ferulic and p-coumaric acids were identified as being present in the heartwood and bark (8, 9). Later salicin, salireposide, salicyl and gentisyl alcohols, p-hydroxybenzoic, p-coumaric, vanillic and syringic acids as well as vanillin were found in hot water

extracts of P. trichocarpa bark. The concentrated aqueous raffinate was found to contain glucose, galactose and a little arabinose. More recently Grabovskis and Kreicberga (27) detected quinic (XLII) and shikimic (XLIII) acids in P. trichocarpa leaves, bark and wood.

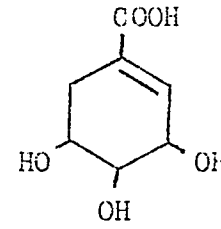
P. grandidentata, like P. trichocarpa, received very little attention until recently. The same products were identified from the alkaline hydrolysate of P. grandidentata wood and bark as were found in P. tremuloides and P. trichocarpa except to different extents (8, 9). Likewise the hot water extractives of P. grandidentata bark contained the same products as P. tremuloides bark. In addition the former species contained vanillic acid which was not present in P. tremuloides bark. Grandidentatin, a new glucoside was isolated from P. grandidentata bark (28) and identified as cis-2-hydroxycyclohexyl-2-O-p-coumaryl-β-D-glucopyranoside (XLIV). Hydrolysis of grandidentatin with calcium hydroxide yield p-coumaric acid and another new glucoside grandidentin (XLV), otherwise called cis-2-hydroxycyclohexyl-β-D-glucopyranoside.



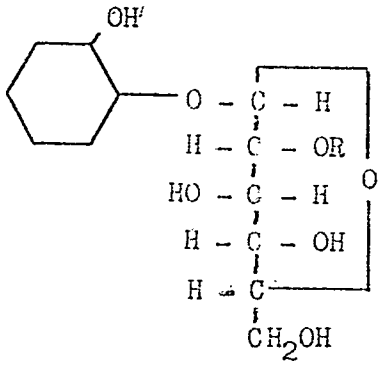
XLI



XLII

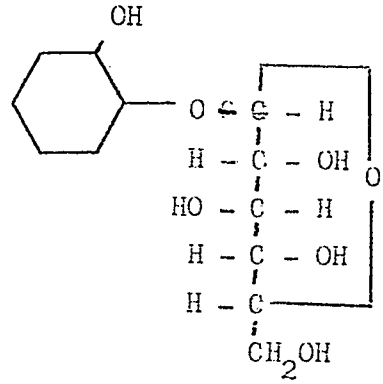


XLIII



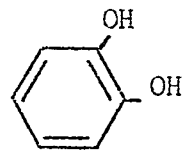
R = p-HO-C$_6$H$_4$CH = CHCO

XLIV



XLV

Pearl, Darling and Justman (20) isolated populin from P. grandidenta leaves but it was probably an artifact formed from tremuloidin by a rearrangement during isolation procedures (24, 25). Further studies on the same leaves (29, 30) resulted in the isolation of benzoic acid, salicin, salicyloyl-salicin benzoate, pyrocatechol (XLVI), tremuloidin and populin.



XLVI

Mamillaria and Opuntia are two genera of the Cactus family (Cactaceae) which grow wild on the Canadian prairies. The Mamillaria (Ball cactus) genus consist of one species, Mamillaria vivipara (Nutt) Haw. (Neomamillaria vivipara (Nutt) Britt. and Rose) (Purple Cactus). The Opuntia (Prickly Pear) genus is composed of two species, Opuntia fragilis (Nutt) Haw. (Brittle Prickly Pear) and Opuntia polycantha Haw. (Prickly Pear).

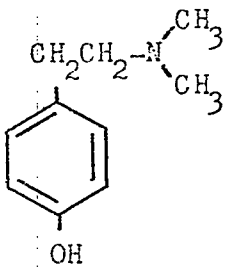
Opuntia fragilis is a low growing, decumbent cactus often found in very large mats, generally red or reddish green in color. The spines are located in divaricate groups and are from one-half to three-quarters of an inch long. The flowers are pale yellow in color and about two inches in width, while the fruit is a fleshy berry approximately one inch long. It is very common on dry prairie throughout drier parts of Southwestern Saskatchewan (1). Many other species of cactus are found elsewhere. They have been studied more thoroughly and will be discussed later.

Since cactus is such a widely known plant and because of its abundance in almost every country many possible uses have been investigated. The earliest research was concentrated on the value of Cactus for Stock food (32, 33, 34, 35, 36). Because of its high mineral content, mainly calcium, magnesium and potassium, and high carbohydrate composition it compares favorably with other green fodders. Later research was directed towards the possible production of ethyl alcohol from the fermentation of Cactus (37-41). In general it was found that better yields of ethanol were obtained from cheaper more readily available materials. The latex from Opuntia vulgaris (42) yields an amber colored gum which resembles plantation smoked sheet rubber in color and Guayule rubber in its properties. Fowler (43) studied the fermentation of prickly pear for the production of artificial manure. Opuntia fulgida contains cholla gum to a very large extent and is

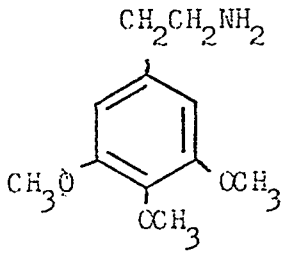
a possible source of carbohydrates (44-47).

Several possible medicinal uses have been found for some of the pharmacologically active constituents of cacti extracts. The flowers of Opuntia vulgaris minus their ovaries have been used in treating enteritis (48). Penfold and Morrison (49) reported that the aqueous extract of the leaves of Opuntia inermis had great curative value for treatment of diabetes. The decoction of Opuntia ficus indica flowers acts as a diuretic presumably due to high potassium content (50). Diacono and Massa (51) found that the calcium-magnesium pectate present in certain cacti decreases clotting and bleeding times. The same compound also prolongs the action of penicillin without anaphylactic shock. It was known that Peyote had a strong pharmacological effect giving a sense of well-being and color visions due to the pharmacological action of the Anhalonium alkaloids.

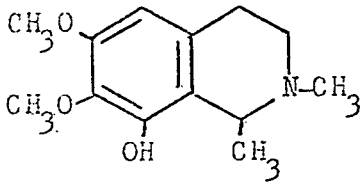
Many alkaloids have been isolated from cactus, the majority of the work involving Anhalonium species. Heffter did most of the initial work on the Anhalonium alkaloids (52), isolating seven bases which he named anhaline (XLVII), mescaline (XLVIII), pellotine (XLIX), anhalonidine (L), anhalonine (LI), anhalamine (LII), and lophophorine (LIII). They are all closely related chemically. Anhaline is identical with hordenine of barley germs, and was known to be p-hydroxyphenylethyldimethylamine. Mescaline was synthetized and shown to be β-3,4,5-trimethoxyphenylethylamine. The composition of the remaining alkaloids was known but proof of structure was not obtained until later.



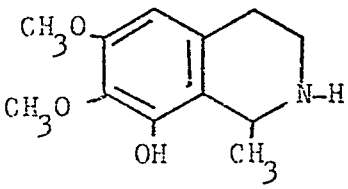
XLVII



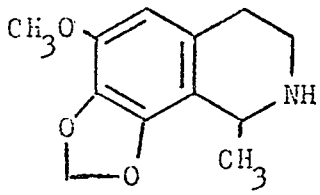
XLVIII



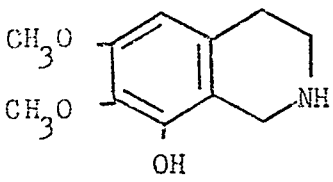
XLIX



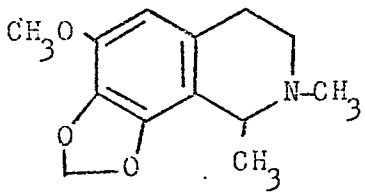
L



LI



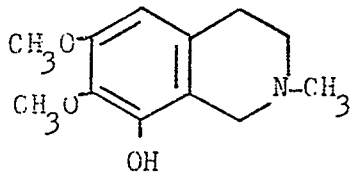
LII



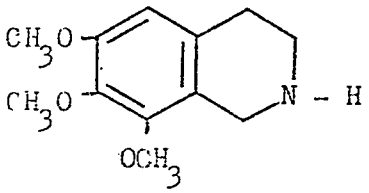
LIII

Späth and coworkers (53, 57) proved the structure of anhalamine to be 7,8-dimethoxy-6-hydroxy-1,2,3,4-tetrahydroisoquinoline by synthesis. Pellotine and anhalonidine were shown by degradative and synthetic means to be structures (XLIX), and (L) respectively (54-56). Späth and Becke (58) synthetized anhalidine (LIV) from anhalamine and showed it to be identical to the naturally occurring alkaloid. Anhalonine and lophophorine were synthetized by Späth and Kesztler (59) and similarly were shown to be the same as the natural alkaloids. Later Späth and Bruck (60, 61, 62) and Späth and Becke (63) isolated and identified several new alkaloids from

mescal buttons as N-methylmescaline, N-acetymescaline, O-methylanhalonidine and anhalinine (LV).



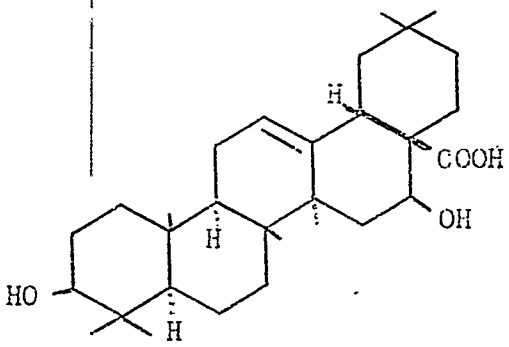
LIV



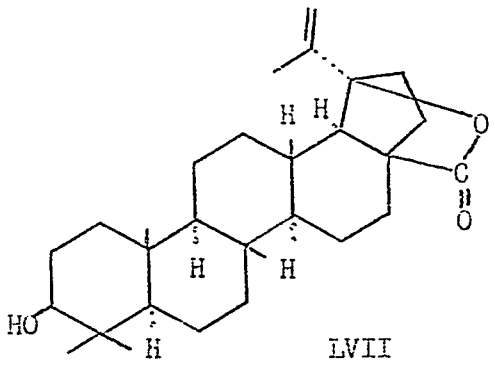
LV

In addition to this work further investigations have been carried out on the same or other species by other workers. Roca (64) working with the large organ cactus of Mexico Pachycereus marginata isolated three alkaloids which were named cereine, pachycerine and ochoterenine; however no attempts at characterization or elucidation of structure were reported. Hordenine and p-hydroxyphenylethyltrimethylammonium hydroxide were isolated from Trichocereus candicans (65). Additional research on Trichocereus candicans by Labriola (66) resulted in the isolation of candicene (p-hydroxyphenyltrimethylammonium hydroxide). Hydroxycandicene (3,4-dihydroxyphenyltrimethylammonium hydroxide) was isolated from Stetsonia coryne. Earlier Reti, Arnolt and Luduena (67) obtained an alkaloid from Cereus coryne Salmm. which they thought was a dihydroxyphenyltrimethylammonium compound presumably related to the substances characterized by Labriola. Castrillon (88) synthetized another alkaloid by the condensation of mescaline with formaldehyde using the Eschweiler-Clarke reaction. The product obtained was 1-methyl-6,7,8-trimethoxy-1,2,3,4-tetrahydroisoquinoline. Studies on Lophophora williamsii by McLaughlin and Paul (68) resulted in the detection of hordenine. In addition to these alkaloids many other constituents have been isolated, and the majority identified.

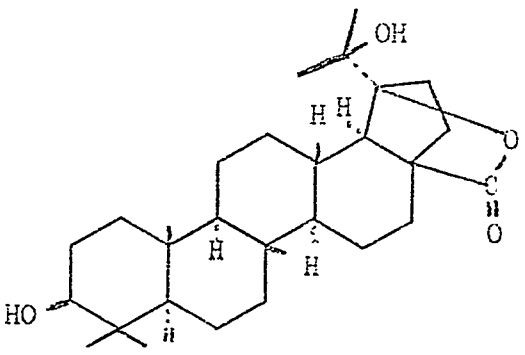
Djerassi, Thomas and Monsimer (69) isolated and identified a triterpene from Myrtillocactus cochal which they called cochalic acid (16 β-hydroxyoleanolic acid) (LVI). Similar studies by Djerassi, Farkas, Liu, and Thomas (70) with Lemaireocereus thurberi and Lemaireocereus stellatus resulted in the discovery of thurberogenin (LVII), and stellatogenin (LVIII).



LVI

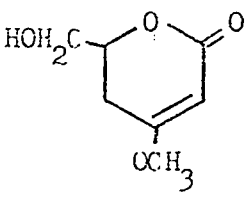


LVII

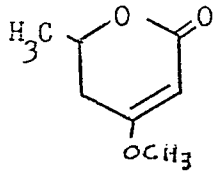


LVIII

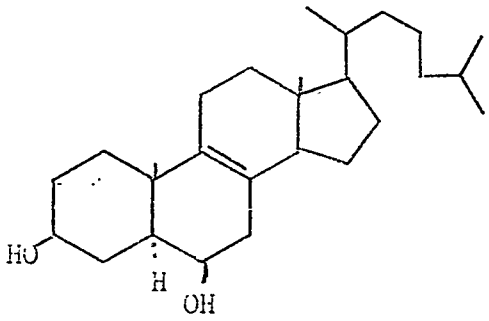
Dawidar and Fayez (71) detected the presence of β-sitosterol in Opuntia ficus indica; Arcoleo, Rucia, and Natoli (72), working with the same species, also isolated β-sitosterol as well as a mixture of the free fatty acids; lauric, myristic, palmitic, stearic and oleic. From Opuntia elatior Ganguly, Govindachari, and Mohamed (73) isolated β-sitosterol as well as opuntiol (LIX), and 2-methyl-4-methoxy-α-pyrone (LX) which they characterized. Djerassi, Murray, and Villotti (74) found a new cactus sterol, peniocerol (LXI) (Cholest-8-ene-3 β -6 α-diol) in Peniocereus fosterianus.



LIX



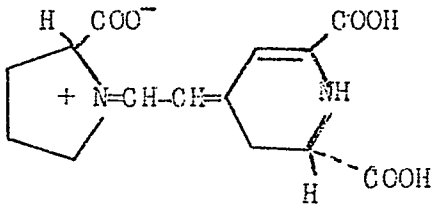
LX



LXI

A great deal of work has been done on Opuntia fulgida, the white cholla cactus (44-47) from which cholla gum is obtained. The gum has been partially degraded to give several oligosaccharides. Parikh and Jones (47) have proposed a structure for the gum, stating that the repeating unit of the polysaccharide consists of a total of thirty-six residues with a backbone of 1 → 6 galactose residues. It is a highly branched structure.

Since cactus often have a purple tinge to their flowers it is not unreasonable to expect the presence of anthocyanin-type compounds. Erkut (75) isolated a red-violet pigment from Opuntia robusta which was mainly betanin with probably traces of prebetanin, isobetanin and isoprebetanin. Indicaxanthin (LXII) has been observed in Opuntia ficus indica (76) and its structure (LXII) has been confirmed. Chlorophyll a and b, carotene, xanthophyll, and a red carotenoid probably rhodaxanthin have been detected in Opuntia humifusa (77).



LXII

Several enzymes have been found in cacti species. Hurdesan (78) discovered that Opuntia vulgaris pulp contained several diastases. Sanwal and Kreshan (79) have detected a specific phosphomonoesterase in various cacti. This enzyme removes orthophosphate from fructose 1,6-diphosphate and β-glycerophosphate. Just recently, materials with glucose-6-phosphatase and fructose 1,6-diphosphate activity have been observed in Opuntia ficus indica (80).

Several flavonoid type compounds and related glucosides have been isolated from cacti. Paris (81) observed the presence of a flavonoside in Opuntia vulgaris, while Reznik (82) isolated quercetin-3-glycoside from several cacti species. Isoquercetin and the glucosides of isorhamnetin and quercetin have been isolated from Opuntia dillenii (83, 85). The presence of anthocyanins and isorhamnetin have also been demonstrated in Opuntia ficus indica (84). The flowers of Opuntia lindheimeri (86) have only recently been shown to possess the 3-galactoside of quercetin; the 3-galactoside, the 3-rutinoside and the 3-rhamnogalactoside of isorhamnetin. Nordal, Gether and Haustveit (87), working with Opuntia ficus indica have found several non-volatile acids, two of which were malic and citric.

II. OBJECTS OF RESEARCH

Populus balsamifera (Balsam poplar) is a very common tree growing in low lying areas throughout the North American continent. The buds and heartwood of this species have been studied quite extensively while considerably less work has been reported on the bark itself. Knowledge of the chemical and morphological composition of a pulpwood species is basic to its efficient and complete utilization. Much can be learned from the origin of materials in explaining the role of the bark in the metabolism of the tree. Since certain elements of bark tissue serve as avenues of translocation of plant metabolites; others as metabolite storage, it was decided to examine the nature of the products as they occur in the bark itself.

Extensive phytochemical studies have been carried out on various species of Cacti and many chemically interesting and pharmacologically active substances have been isolated and characterized. Typical examples are the Anhalonium alkaloids and mescaline. A systematic phytochemical study of Opuntia fragilis has not been reported. As Opuntia fragilis is a common plant which thrives on dry prairie throughout southwestern Saskatchewan, it was thought that a detailed investigation of the chemical constituents of this cactus was warranted.

III. DISCUSSION

(A) Extractives from Populus balsamifera

A white amorphous solid (A), which gradually darkened on exposure to air, precipitated when the acetone extract of Populus balsamifera bark was cooled. The infrared spectrum exhibited strong bands at 1735 cm^{-1} (attributable to $\rangle C = O$ stretching) and at 2920 and 2860 cm^{-1} due to C - H stretching modes, a weak band at 1377 cm^{-1} due to C - H deformation frequency for a methyl group, a strong band at 1460 cm^{-1} due to vibration of methylene groups, a strong doublet at 724 and 714 cm^{-1} which is associated with skeletal vibrations of methylene chains $-(CH_2)_n-$ as well as bands at 1270 and 1160 cm^{-1} which are suggestive of band progression in long chain aliphatic compounds with a chain length of at least twenty-two carbon atoms (89). In addition there was a medium intensity band at 3500 cm^{-1} probably due to water of crystallization since it was found in the present work that such unsaturated esters held onto their water of crystallization very strongly, acting as hydrophilic reagents. A medium intensity band at 1625 cm^{-1} was present, suggesting unsaturation. There also appeared a strong band at 1710 cm^{-1} presumably due to the $\rangle C = O$ stretching of an ester carbonyl group appearing at a lower frequency than the $\rangle C = O$ frequency for a saturated ester (1735 cm^{-1}) due to the presence of unsaturation or else the presence of free acids in the sample. The conclusion from this spectral study was that material (A) could be a mixture of long chain saturated and unsaturated aliphatic esters.

It is not surprising that long chain esters, if present, would precipitate out of the acetone solution on cooling as a low solubility would be expected due to the large molecular weight of such compounds. A sodium fusion test showed the absence of nitrogen, sulfur and halogens.

Concentration of the acetone mother liquor remaining after the removal of A gave a dark brown semi-solid (B) which was soluble in hot 5% aqueous sodium hydroxide and practically insoluble in 5% aqueous sodium bicarbonate. This material gave a dark purple colour with ferric chloride solution, indicative of phenolic material.

Thin layer chromatography (TLC) was tried as a method of purifying material (A). Many solvent systems were investigated using Silica gel G plates (20 x 20 cm) 0.1 to 1.6 mm. in thickness. The spots obtained were visible under ultra-violet light or were made visible by spraying the plates with 0.1 N potassium permanganate and concentrated sulfuric acid. It was found using Silica gel G plates 1.3 mm. in thickness and benzene: dioxan: glacial acetic acid (90:25:4) as the developing solvent that A could be separated into five fractions which fluoresced blue under ultra-violet light. On the assumption that this was a satisfactory separation, preparative thin layer chromatography was performed under the above conditions. The five fractions were collected separately from the plates and each fraction was extracted from the silica gel using a small Soxhlet extractor. An infrared spectrum of each fraction showed that they were all similar, except that fractions 1 and 2 showed very weak absorption in regions corresponding to unsaturation, while fractions 3, 4 and 5 showed quite strong evidence of unsaturation. It appeared that enrichment of the unsaturated fatty ester portion was taking place. This observation was supported by the gas-liquid chromatographic behavior of the methyl esters (obtained as described below by hydrolysis followed by methylation of the acids) which showed that fractions 4 and 5 contained a much greater percentage of unsaturated esters than fractions 1 and 2. Rechromatography of

each of fractions 1 to 4 gave rise to more than one spot under the same conditions used initially. Melting point determinations showed that the melting point decreased as the R_f increased. These results suggested that these esters were separating out in groups of similar chain length. One would expect a higher melting point for an ester of longer chain length than one with a shorter chain length.

If these compounds were indeed esters as was suspected one should be able to hydrolyze them and identify the component acids and alcohols. The material from the initial acetone extraction (A) was hydrolyzed using 5% aqueous sodium hydroxide. The hydrolysis mixture was extracted with ether from which was obtained a white solid which was further extracted with Skelly B to remove any unchanged esters, and recrystallized from ethanol to give a product m.p. 82-83°. Treatment of the aqueous solution is described later. The infrared spectrum of the material, m.p. 82-83° showed a strong band at 3400 cm^{-1} due to OH, two strong bands at 2920 and 2860 cm^{-1} due to C-H stretching modes, a strong band at 1460 cm^{-1} suggestive of methylene vibrations, a weak band at 1377 cm^{-1} due to a terminal methyl group, a medium band at 1050 cm^{-1} due to a primary alcohol group and a doublet at 724 and 714 cm^{-1} characteristic of long chain aliphatic compounds. This spectrum is indicative of a long chain saturated aliphatic alcohol. This spectrum was almost identical to one of an authentic sample of stearyl alcohol. Since there was a complete absence of unsaturation, the unsaturation present in material A must be in the acid part of the ester.

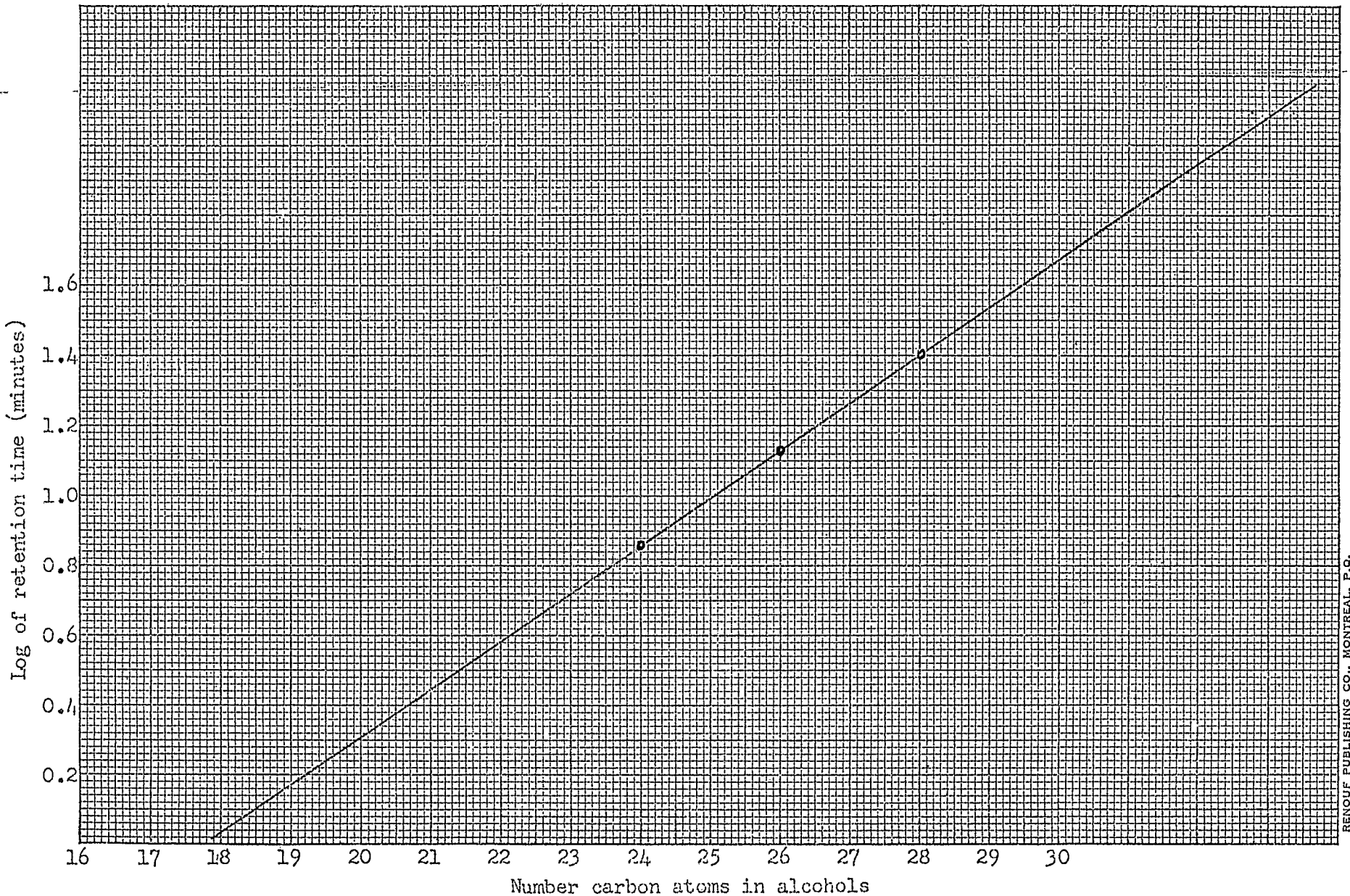
The aqueous solution from the hydrolysis was acidified, and extracted with ether to give a white solid, m.p. 78-80°. Its infrared spectrum

showed a medium intensity band at 3400 cm^{-1} probably associated with the hydroxyl group of a carboxyl function, two strong bands at 2920 and 2860 cm^{-1}, a strong band at 1700 cm^{-1} due to $>C = O$ of a carboxyl group, several weak bands between 1400 and 1100 cm^{-1}, a weak band at 1050 cm^{-1}, and a doublet at 724 and 714 cm^{-1}. There appeared to be some evidence of unsaturation but the absorption was very weak compared to that of saturated bands.

Isolation of these acids and alcohols showed that the original material (A) was indeed a mixture of long chain esters as suggested. It was known (21) that normal long chain saturated aliphatic alcohols can be separated by GLC on a column of SE-30 on glass beads. At a column temperature of 245° the unknown alcohols from the hydrolysis of A were separated successfully. A standard curve was prepared using normal saturated aliphatic alcohols of known chain length (C_{24}, C_{26} and C_{28}) by plotting the number of carbon atoms in the alcohols against the log of their retention times. (FIGURE 1).

Long chain fatty acids (those above C_{12}) are usually gas-chromatographed in the form of their methyl esters. A column of butan-1,4-diol succinate on acid washed celite 545 has been used (21) to separate both saturated and unsaturated esters. This particular column separates methyl esters on the basis of their chain length as well as their degree of unsaturation. The acids obtained from the hydrolysis of A were methylated with methanolic sulfuric acid. At a column temperature of 220° the methyl esters were found to separate satisfactorily. A known mixture of saturated (16:0 palmitic, 18:0 stearic, 20:0 arachidic, 22:0 behenic, 24:0 lignoieric, 26:0 cerotic) and unsaturated (16:1 palmitoleic, 18:1 oleic, 18:2 linoleic,

FIGURE 1
STANDARD CURVE FOR ALCOHOLS



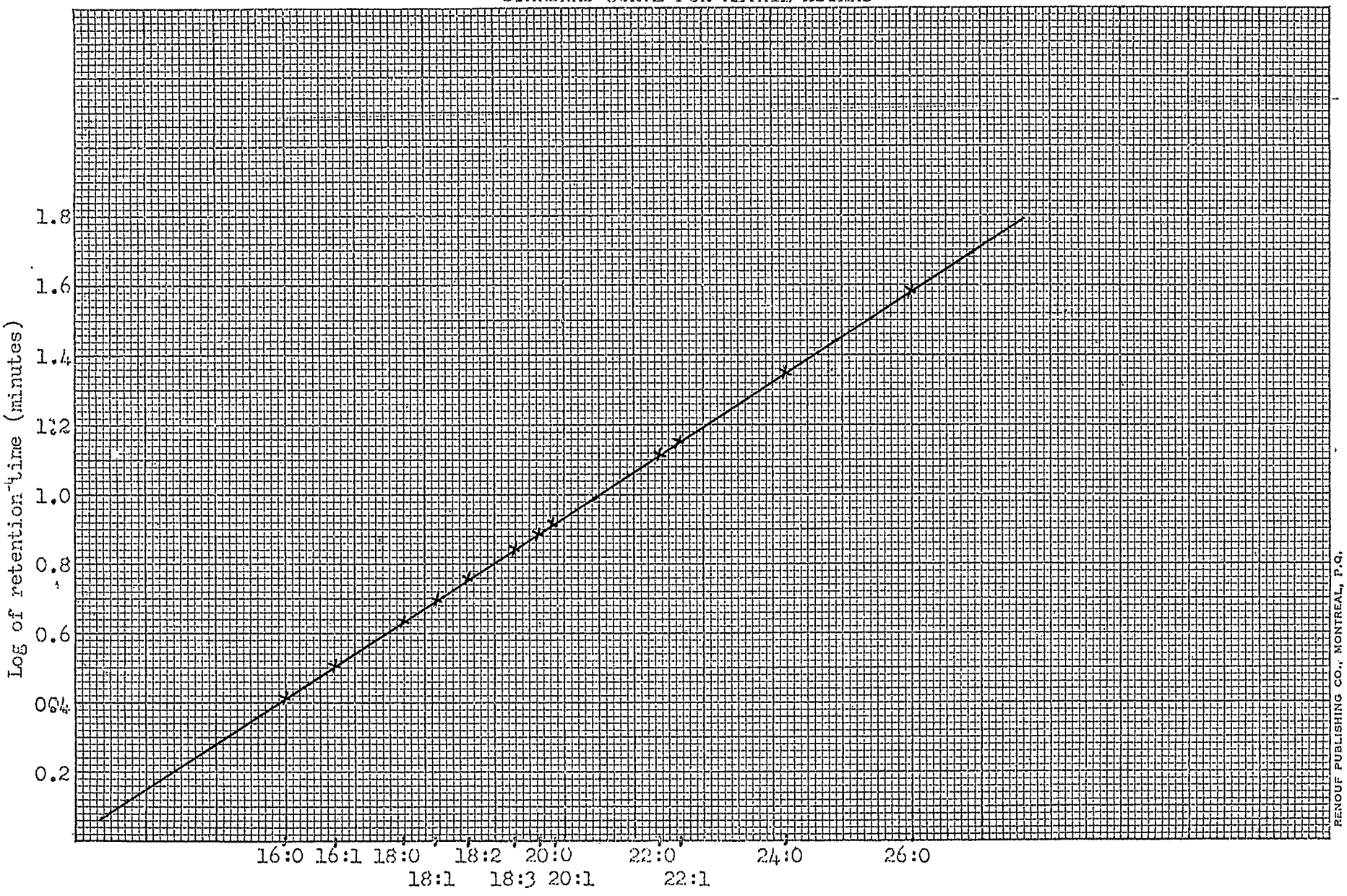
18:3 linolenic, 20:1 arachidonic, 22:1 erucic) methyl esters from methylated rapeseed oil was used to prepare the standard curve (FIGURE 2).

The five fractions obtained from preparative thin layer chromatography were each hydrolyzed with aqueous sodium hydroxide; the resulting acids and alcohols were separated by conventional means and the acids were methylated. The alcohols were gas-chromatographed under the conditions used to prepare the standard curves previously referred to. The log of the retention time was calculated for each component and the lengths of the unknown alcohols were deduced from the standard curve. The amount of each alcohol was calculated from the area under the peak using the triangulation method.

Likewise the methyl esters were gas-chromatographed at the same temperature and using the same column as used for the separation of the standard methyl esters. The log of the retention time was calculated for each component of the mixture and the length of the methyl ester as well as the degree of unsaturation was deduced from the standard curve. The amount of each methyl ester was calculated from the area under the peak. From the results obtained one can see that the amount of short chain acids (Table 15) and alcohols (Table 14) increase as one goes from fraction 1 with the lowest R_f value to fraction 5 with the highest R_f value. Similarly, the percentages of long chain acids and alcohols decrease as one goes from fraction 1 to fraction 5. This observation would again suggest, as was previously postulated, that the mixture of esters was separated into groups of similar chain length when separated by preparative thin-layer chromatography. Also it was evident from these results that the same acids and alcohols were present to different extents in each of the five fractions.

FIGURE 2

STANDARD CURVE FOR METHYL ESTERS



It can be easily visualized that an appreciable number of esters could be present in each of the five fractions obtained from A, since there are large numbers of combinations of different acids and alcohols possible which would give rise to isomeric esters.

To test the hypothesis that the ester components of fraction A separated into groups of similar chain length, several known esters of known chain lengths were synthetized. These esters were chromatographed (TLC) under the same conditions as those used in the preparative thin layer chromatography of A. The results showed that the known esters did indeed separate according to chain length, the longer the chain length the lower the R_f value. It was also found that esters of the same chain length, but composed of different acid and alcohol fragments $CH_3(CH_2)_mCO_2(CH_2)_nCH_3$ and $CH_3(CH_2)_nCO_2(CH_2)_mCH_3$ (such as stearyl arachidate and cetyl behenate), could not be resolved under these conditions. These results confirmed that the esters present in A separated in groups according to chain length in preparative thin layer chromatography.

The long chain saturated and unsaturated aliphatic esters isolated in this study have probably been obtained before from other poplar species as P. grandidentata and P. tremuloides (9). No previous attempt was made, however to separate and identify the individual esters. The mixtures of esters were hydrolyzed and the component acids and alcohols were identified. All the saturated acids $C_1 - C_{10}$ and $C_{12} - C_{28}$ have been identified previously. Free fatty acids as well as esters have been shown to exist in the heartwood and the bark of P. tremuloides, P. grandidentata and P. tacamahaca (7). In addition, the unsaturated acids oleic, linoleic, linolenic and arachidonic have been identified previously. The only alcohols reported (19, 21) so far have been glycerol, tetracosanol, hexacosanol,

heptacosanol and octacosanol. It is interesting to note that in the present study, stearyl, eicosyl, docosyl and pentacosyl alcohols were also found in addition to those already reported found in other species. However they are present to a very small extent compared to the amounts of tetracosanol, hexacosanol and octacosanol found.

Phenols usually occur in nature as phenolic glycosides and esters. The phenolic material (B) obtained as described earlier was hydrolyzed using aqueous sodium hydroxide. The free phenolic material was isolated in the usual way. Pearl and his coworkers (8, 9) who have done a considerable amount of work on some species of the Populus genus always separated the phenolic acids, ketones and aldehydes, obtained after hydrolysis, by paper chromatography using the solvent systems and spray reagents which they appear to have perfected. They extracted the individual spots from the paper and identified each individual component by comparing its ultraviolet absorption spectrum with that of an authentic sample. The amount of each component present was obtained from a standard curve prepared by plotting the optical density of known compounds versus their concentration. This appeared to be a sound process but involved a considerable amount of work.

It is known (90) that methylation of phenols improves their properties for gas-liquid chromatographic analysis. With this purpose in mind the phenolic material obtained after hydrolysis of B was methylated with diazomethane. The product was gas-chromatographed on a column composed of silicone 550 oil on acid-washed firebrick.

Chromatographic analysis of the methylated material under these conditions gave rise to thirteen peaks with retention times 1.14, 1.44, 1.92, 2.60, 3.62, 4.56, 4.96, 6.78, 7.62, 10.12, 13.06, 18.70 and 33.14 minutes,

respectively. Each one of these thirteen peaks was collected directly onto potassium bromide as the compound emerged from the column (using the method described by Snavely and Grasselli (91).

Several authentic phenolic acids, aldehydes and ketones previously found in a number of species of the Populus genus, as well as others which were thought to be possible constituents were also methylated and gas-chromatographed under conditions identical to those given above. Again each one of these known compounds was collected directly on potassium bromide (91) and an infrared spectrum was obtained. The infrared spectra of some of the unknown compounds from the methylated mixture were characteristic of long chain saturated aliphatic esters. A standard curve was prepared using methyl palmitate, methyl stearate and methyl arachidate by plotting the log of the retention times versus number of carbon atoms in the methyl esters. Examination of the infrared spectra of the standards, and the unknowns, a comparison of retention times of the standards and the unknowns, and reference to the standard curve of long chain saturated aliphatic esters revealed the identity of eleven of the thirteen components present in the unknown methylated mixture.

The unmethylated material obtained after hydrolysis of B was chromatographed on Whatman No. 1 paper using n-heptane: n-butylether : water (6:1:1) as solvent. This solvent system was used to separate p-hydroxybenzaldehyde, vanillin and syringaldehyde (92). When the unknown material was developed under these conditions and the paper sprayed with 2,4-dinitrophenylhydrazine spray reagent no yellow spots were obtained, whereas the chromatogram of a mixture of these three aldehydes produced yellow spots when sprayed with the same reagent. These findings agree with the gas-liquid chromatographic results and confirm that no free phenolic aldehydes

Table 1

Acidic Components of hydrolysed acid fraction (B)

Fraction	Retention time (min.) of methyl deriv.	% of total	Identity of acid
1	1.14	0.91	Caprylic
2	1.44	1.15	Pelargonic
3	1.92	0.30	Capric
4	2.60	19.95	p-Hydroxybenzoic
5	3.62	4.87	Tridecylic
6	4.56	3.90	-----
7	4.96	7.69	Vanillic
8	6.78	21.93	p-Coumaric
9	7.62	6.96	-----
10	10.12	2.92	Palmitic
11	13.06	18.20	Ferulic
12	18.70	5.22	Stearic
13	33.14	6.19	Arachidic

or ketones are present in P. balsamifera bark. These results are also in agreement with previous findings (9) that phenolic acids are the chief product of alkaline hydrolysis of P. tremuloides, P. grandidentata and P. heterophylla barks.

Pearl and his coworkers (9) also isolated vanillin, syringaldehyde, p-hydroxybenzaldehyde, acetovanillone, acetosyringone and syringic acid after alkaline hydrolysis of P. balsamifera bark. These aldehydes and ketones were probably degradation products of the lignin. The method of

isolation used in this study would not permit such products to be formed. Pearl and his coworkers (9) also detected several spots on paper chromatograms which they thought were possibly due to aliphatic acids. These are probably similar to the short chain acids identified in this study.

The presence of both phenolic acids and aliphatic acids in B supports the previous statement that B was a mixture of phenolic esters.

(B) Extractives from Opuntia fragilis.

The powdered cactus was defatted using light petroleum (b.p. 60-80°) and extracted with hot ethanol. On cooling, a white amorphous solid (C) precipitated. Its infrared spectrum indicated it consisted of long chain aliphatic esters as suggested by the strong absorptions at 3400, 2920, 2860, 1735, 1460, 724 and 714 cm^{-1}. A sodium fusion (Lassaigné) test indicated the absence of nitrogen, sulfur and halogens in C.

The ethanol mother liquor was concentrated and gave a dark green oily semi-solid (D) which did not contain nitrogen. However this same material gave a purple color with ferric chloride indicative of phenolic material.

Since alkaloids frequently occur in nature as salts of simple organic acids such as oxalic, malonic and succinic acid, it was thought advisable to basify the powdered cactus, and extract it with chloroform in the hope that alkaloids, if present, would be obtained. The cactus, after extraction with ethanol, was basified with ammonium hydroxide and extracted with chloroform. A dull yellow amorphous solid was obtained from the chloroform solution and, on recrystallization from benzene, a white crystalline, nitrogen containing solid (m.p. 75-76°) resulted. The infrared spectrum of this compound was indicative of an amide with strong absorption at 3350, 3150, and 1670 cm^{-1}. This compound analyzed for C_2H_5NO and was quickly shown to be acetamide by comparison with an authentic sample. Acetamide may result from ammonolysis of an ester or by reaction of ammonia with free acetic acid present in the cactus to give ammonium acetate which on distillation is known to yield acetamide (94).

The white amorphous solid C was chromatographed on a column of

alumina using the following solvent systems:

1) Skelly F: A white waxy solid was obtained (m.p. 73-74°) which was probably a mixture of wax hydrocarbons as indicated by the strong methylene and methyl absorptions in the infrared. One would expect these hydrocarbons to be eluted first with a non-polar solvent such as Skelly F. No attempt was made to identify the components of this mixture although it is known (21) that they can be characterized quite readily by gas-chromatography.

2) Skelly F/benzene (3:1)

3) Skelly F/benzene (1:1)

4) Skelly F/benzene (1:3)

The infrared spectra of the fractions obtained using these three solvent systems were the same showing strong absorptions in the infrared at 2920, 2860, 1730, 1460, 1170, 724 and 714 cm^{-1}, which are characteristic of long chain saturated aliphatic esters as described earlier. These fractions were combined as (E).

5) Benzene

6) Benzene/ether (1:1)

The two fractions which were eluted using these developing solvents did not have sharp peaks in the infrared probably due to the fact that they are not pure. They appeared to be saturated aliphatic esters but were not examined further.

7) Ether/ethanol (1:1)

A white amorphous solid (F) was obtained with this solvent system with absorptions in the infrared at 3400, 2920, 2860, 1700, 1625, 1585, 1500, 1460, 1260, 1160, 1020, 800, 724 and 714 cm^{-1}. The spectrum appeared to

indicate either an unsaturated long chain aliphatic ester or an α, β-unsaturated ketone. However an attempt to prepare a 2,4-dinitrophenylhydrazone failed, which suggested that this compound was probably an unsaturated ester.

8) Ethanol

A very small amount of a white solid similar to that isolated in fraction 7 was obtained.

9) Acetic acid (5%).

The column was eluted with acetic acid in an attempt to isolate the material not recovered. Large quantities of aluminum acetate were obtained which, when basified and extracted with ether, gave only acetic acid.

Another column was also developed using alkaline alumina (Brockman activity 1) using the same solvent systems. The same products were isolated except that the saturated esters were obtained to a much lesser degree than from the previous column. In this case, long chain saturated aliphatic alcohols (C_{22}, C_{24}, C_{26}, C_{28}) were isolated which suggests that this alumina was alkaline enough to cause hydrolysis of the saturated esters. The column was not eluted with acetic acid which would have supposedly eluted the acids resulting from the hydrolysis of the saturated esters which would be strongly absorbed on the column.

The saturated aliphatic ester fraction (E) was hydrolyzed with alcoholic potassium hydroxide. The component acids and alcohols were isolated in the usual manner, and once again the acids were methylated. The alcohols and methyl esters were gas-chromatographed and the identity of the alcohols and methyl esters were determined from the standard curves prepared for this purpose. The straight chain alcohols (C_{20}, C_{22}, C_{24}, C_{26}, C_{28}, C_{30})

were identified. The normal acids (C_{16}- C_{22}) were present as their methyl esters. These results showed that fraction (E) was a mixture of long chain saturated aliphatic esters.

These results are very informative, but do not give any indication as to the total length of the individual esters or which component acid and alcohol are present in which ester. It is generally recognized that naturally occurring waxes usually consist of mixtures of homologous components whether they be esters, acids, alcohols, ketones or hydrocarbons (93, 94). It seemed likely that this fraction (E) was similarly constituted; use was therefore made of mass spectrometric measurements to extend and complete the chemical analysis of the wax. The mass spectrum of recrystallized (E) indeed showed that this mixture was a homologous series of saturated aliphatic esters. Ten parent peaks differing by fourteen atomic mass numbers (CH_2) were observed (Table 2).

Before these results can be interpreted an understanding of the fragmentation mechanism is necessary, or should be established by examination of the organic ions. Mass spectroscopy of long esters is discussed quite extensively by Ryhage and Stenhagen (95). A fragmentation mechanism is outlined for long chain saturated aliphatic esters.

$$CH_3(CH_2)_m - O - \underset{\underset{O}{\|}}{C} - (CH_2)_n CH_3$$

$\downarrow$

(a) $\left[C_mH_{2m}\right]^+$

(b) $\left[CH_3(CH_2)_m - O - \underset{\underset{OH}{|}}{C} = CH_2\right]^+$

(c) $\left[HO - \underset{\underset{OH}{\|}}{C} - (CH_2)_n CH_3\right]^+$

(d) $\left[\underset{\underset{O}{\|}}{C} - (CH_2)_n CH_3\right]^+$

(e) $\left[(H_2C)_m - O - \underset{\underset{O}{\|}}{C} - (CH_2)_n CH_3\right]^+$

Table 2

Composition of Fraction E

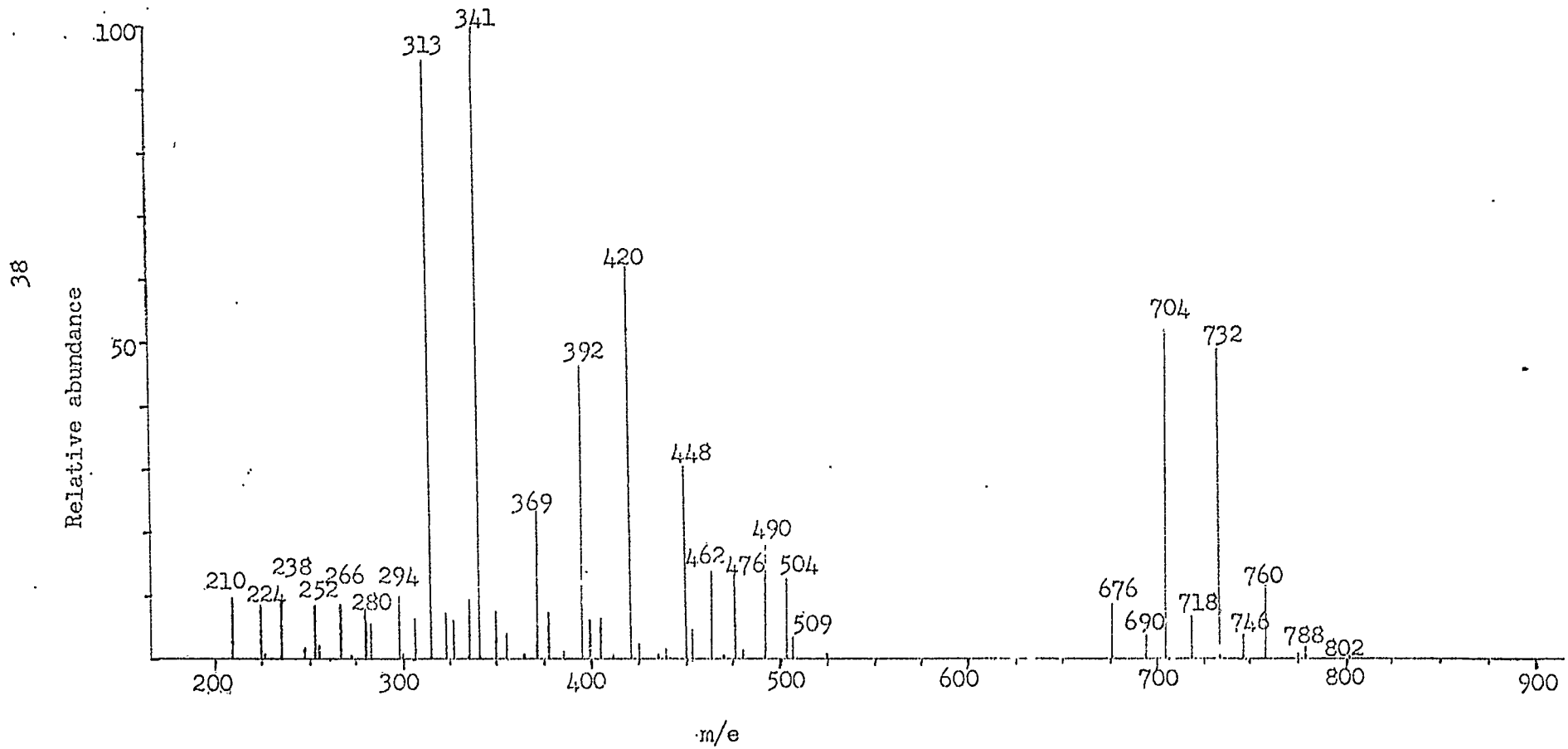
M^+	Corresponding Molecular Formula	Relative* abundance
676	$C_{46}H_{92}O_2$	8.76
690	$C_{47}H_{94}O_2$	3.64
704	$C_{48}H_{96}O_2$	53.50
718	$C_{49}H_{98}O_2$	7.02
732	$C_{50}H_{100}O_2$	49.00
746	$C_{51}H_{102}O_2$	3.92
760	$C_{52}H_{104}O_2$	11.20
774	$C_{53}H_{106}O_2$	1.12
788	$C_{54}H_{108}O_2$	1.96
802	$C_{55}H_{110}O_2$	0.56

* Relative to base peak at m/e 341 as 100.

One can see from this fragmentation mechanism that the alcohol portion will give rise to fragments of even mass numbers and the fragments derived from the acid portion will have odd mass numbers. The most prominent peaks in long chain esters are usually those containing oxygen (95). These ions and fragments listed in the above Scheme are formed in various ways resulting from cleavage of the chain and rearrangement of hydrogen atoms. Fragment (a) arises from expulsion of part of the chain with loss of a hydrogen atom; similarly, fragment (b) results from cleavage of the chain with rearrangement of hydrogen atoms; fragment (c) is caused by alkyl oxygen fission with rearrangement of two hydrogen atoms, while the acylium ion, fragment (d), is formed by loss of the alkoxyl group. Fragment (e) is formed as the result

Figure 3

Portion of mass spectrum of Fraction E



of simple cleavage of the chain.

With such an understanding of the fragmentation mechanism, a systematic study of the spectrum itself can be made. Inspection of the spectrum showed that the major fragments occurred at m/e 285, 313, 341, 369, 392, 420, and 448. There are also many other fragments of lesser intensity.

The alcohol fragments (even mass numbers) were first studied and are listed in Table 3.

As illustrated in this table some of the fragments were quite weak in intensity while others were very strong. The C_mH_{2m} fragments were usually the most intense and this appears to be the key fragment for the alcohol components. It is known that hydrocarbon peaks such as these are intense (96). However, cleavage of this chain may occur further with loss of one or more methylene groups from C_mH_{2m}, which would result in a significant contribution to lower members in the C_mH_{2m} series. This must be taken into account in quantitative estimations.

The acid fragments (odd mass numbers) were examined. The results are illustrated in Table 4.

Using the results from Tables 3 and 4, acid and alcohol fragments can be combined to fit the mass numbers found for the esters in the homologous series (m/e 676-802). Completion of this procedure suggested the following esters as possibilities for the parent mass numbers (Table 5).

Table 3

Alcohol fragments of fraction E

m	$[C_mH_{2m}]^+$ M+	Relative abundance	$[CH_3(CH_2)_m-O-\overset{OH}{\overset{|}{C}}=CH_2]^+$ M+	Relative abundance	$[CH_3(CH_2)_m-O-\overset{O}{\overset{\|}{C}}]^+$ M+	Relative abundance
15	210	9.40	284	1.97	269	3.64
16	224	8.60	298	1.66	283	2.80
17	238	9.52	312	20.70	297	11.70
18	252	8.40	326	2.55	311	5.05
19	266	8.40	340	26.2	325	1.96
20	280	7.85	354	1.66	339	3.90
21	294	10.19	368	7.02	353	1.68
22	308	6.45	382	1.40	367	1.68
23	322	7.30	396	2.80	381	2.50
24	336	9.52	410	1.40	395	1.66
25	350	7.30	424	2.52	409	2.80
26	364	1.12	438	3.92	423	1.66
27	378	7.60	452	3.64	437	10.10
28	392	46.50	466	2.52	451	3.08
29	406	6.74	480	5.30	465	5.60
30	420	62.6	494	2.80	479	14.30
31	434	0.98	508	14.50	493	8.12
32	448	30.80	522	1.68	507	39.20
33	462	14.00	536	1.40	521	3.64
34	476	13.40	550	0.56	535	3.08
35	490	18.50	564	0.84	549	1.66
36	504	12.60			563	0.84

TABLE 4

Acid fragments of fraction E

n	$[CH_3(CH_2)_n-\overset{OH}{\overset{|}{C}}=OH]^+$		$[CH_3(CH_2)_n-\overset{O}{\overset{\|\|}{C}}]^+$		$[CH_3(CH_2)_n-\overset{O}{\overset{\|\|}{C}}-O(CH_2)_2]^+$	
	M^+	Relative abundance	M^+	Relative abundance	M^+	Relative abundance
12	229	1.00	211	6.60	255	2.24
13	243	1.20	225	6.10	269	3.36
14	257	2.24	239	6.70	283	2.80
15	271	0.56	253	6.20	297	1.70
16	285	5.10	267	6.20	311	5.05
17	299	1.10	281	5.05	325	1.96
18	313	95.00	295	10.01	339	3.90
19	327	6.20	309	5.60	353	1.68
20	341	100.00	323	9.00	367	1.68
21	355	3.90	337	5.60	381	2.50
22	369	23.60	351	6.70	395	1.66
23	383	1.68	365	7.85	409	2.80
24	397	6.18	379	9.00	423	1.66
25	411	0.56	393	17.80	437	10.10
26	425	2.52	407	5.60	451	3.08
27	439	1.40	421	23.00	465	5.60
28	453	4.50	435	5.32	479	14.30
29	467	0.79	449	12.30	493	8.12
30	481	1.66	463	7.02	507	39.20
31	495	1.12	477	12.30	521	3.64
32	509	3.36	491	8.12	535	3.08
33	523	0.56	505	6.16	549	1.66

TABLE 5

Possible Structures for Parent Esters

m/e 676 $C_{15-32}H_{31-65}-O-\overset{O}{\overset{\|}{C}}-H_{61-27}C_{30-13}$

m/e 690 $C_{15-33}H_{31-67}-O-\overset{O}{\overset{\|}{C}}-H_{63-27}C_{31-13}$

m/e 704 $C_{15-34}H_{31-69}-O-\overset{O}{\overset{\|}{C}}-H_{65-27}C_{32-13}$

m/e 718 $C_{15-35}H_{31-71}-O-\overset{O}{\overset{\|}{C}}-H_{67-27}C_{33-13}$

m/e 732 $C_{15-36}H_{31-73}-O-\overset{O}{\overset{\|}{C}}-H_{69-27}C_{34-13}$

m/e 746 $C_{16-36}H_{33-73}-O-\overset{O}{\overset{\|}{C}}-H_{69-29}C_{34-14}$

m/e 760 $C_{17-36}H_{35-73}-O-\overset{O}{\overset{\|}{C}}-H_{69-31}C_{34-15}$

m/e 774 $C_{18-36}H_{37-73}-O-\overset{O}{\overset{\|}{C}}-H_{69-33}C_{34-16}$

m/e 788 $C_{19-36}H_{39-73}-O-\overset{O}{\overset{\|}{C}}-H_{69-35}C_{34-17}$

m/e 802 $C_{20-36}H_{41-73}-O-\overset{O}{\overset{\|}{C}}-H_{69-37}C_{34-18}$

Fraction E was hydrolyzed as previously mentioned. The alcohols C_{20}, C_{22}, C_{24}, C_{26}, C_{28} and C_{30} (Table 21) and the acids, C_{16}-C_{22} inclusive (Table 22) were identified by gas chromatography. The relative amounts of these component acids and alcohols as determined by mass spectroscopy and gas-chromatography are shown in Tables 3, 4, 21 and 22. Some of the acids and alcohols shown to be present by mass spectroscopy were not detected in the gas-chromatographic results. These acids and alcohols were present in relatively small amounts. Inspection of Table 5 (possible parent esters) together with a check to see if the acid and alcohol fragments which comprise the ester were found in the gas-chromatographic analysis of the

hydrolysis products of E was made. If the acid and alcohol were both shown to be present by means of GLC then it can be said that a particular ester, $CH_3(CH_2)_m-O-\overset{O}{\overset{\|}{C}}-(CH_2)_nCH_3$ may well be present and is composed of a certain acid ($CH_3(CH_2)_nCO_2H$) and alcohol ($CH_3(CH_2)_mOH$). Following this approach the following esters were assumed to be present in the saturated aliphatic ester fraction (E) (Table 6).

TABLE 6

Possible component esters of fraction E.

m/e 676	m/e 690
$C_{30}H_{61}O_2C\ H_{31}C_{15}$	$C_{30}H_{61}O_2C\ H_{33}C_{16}$
$C_{28}H_{57}O_2C\ H_{35}C_{17}$	$C_{28}H_{57}O_2C\ H_{37}C_{18}$
$C_{26}H_{53}O_2C\ H_{39}C_{19}$	$C_{26}H_{53}O_2C\ H_{41}C_{20}$
$C_{24}H_{49}O_2C\ H_{43}C_{21}$	
m/e 704	**m/e 718**
$C_{30}H_{61}O_2C\ H_{35}C_{17}$	$C_{30}H_{61}O_2C\ H_{37}C_{18}$
$C_{28}H_{57}O_2C\ H_{39}C_{19}$	$C_{28}H_{57}O_2C\ H_{41}C_{20}$
$C_{26}H_{53}O_2C\ H_{43}C_{21}$	
m/e 732	**m/e 746**
$C_{30}H_{61}O_2C\ H_{39}C_{19}$	$C_{30}H_{61}O_2C\ H_{41}C_{20}$
$C_{28}H_{57}O_2C\ H_{43}C_{21}$	* $C_{28}H_{57}O_2C\ H_{45}C_{22}$
m/e 760	**m/e 774**
$C_{30}H_{61}O_2C\ H_{43}C_{21}$	* $C_{30}H_{61}O_2C\ H_{45}C_{22}$
* $C_{28}H_{57}O_2C\ H_{47}C_{23}$	

m/e 788	m/e 802
* $C_{30}H_{61}O_2C\ H_{47}C_{23}$	* $C_{30}H_{61}O_2C\ H_{49}C_{24}$
* $C_{28}H_{57}O_2C\ H_{51}C_{25}$	

The starred (*) esters were not unambiguously identified or confirmed as being present as the acidic components were not found in the gas-chromatographic analysis of the methyl esters. The relative abundance of the parent esters m/e 774, 788 and 802 were very small so that it appears that these parent esters are present in only trace amounts. The mass spectrum shows peaks of sufficient intensity at m/e 355 ($C_{22}H_{45}CO_2H_2^+$), m/e 369 ($C_{23}H_{47}CO_2H_2^+$), m/e 397 ($C_{25}H_{51}CO_2H_2^+$) to warrant the suggestion for the existance of these esters. The only reason which can be offered to explain the absence of these acids in the gas-chromatographic analysis is that the mass spectrometer is a more sensitive instrument and is able to detect smaller amounts of these components than is the gas-chromatograph.

A mass spectral analysis was similarly performed on the unsaturated ester fraction (F). A homologous series of twelve esters was evident from the mass spectrum with parent peaks differing by one methylene unit unit (Table 7).

TABLE 7

Composition of fraction F.

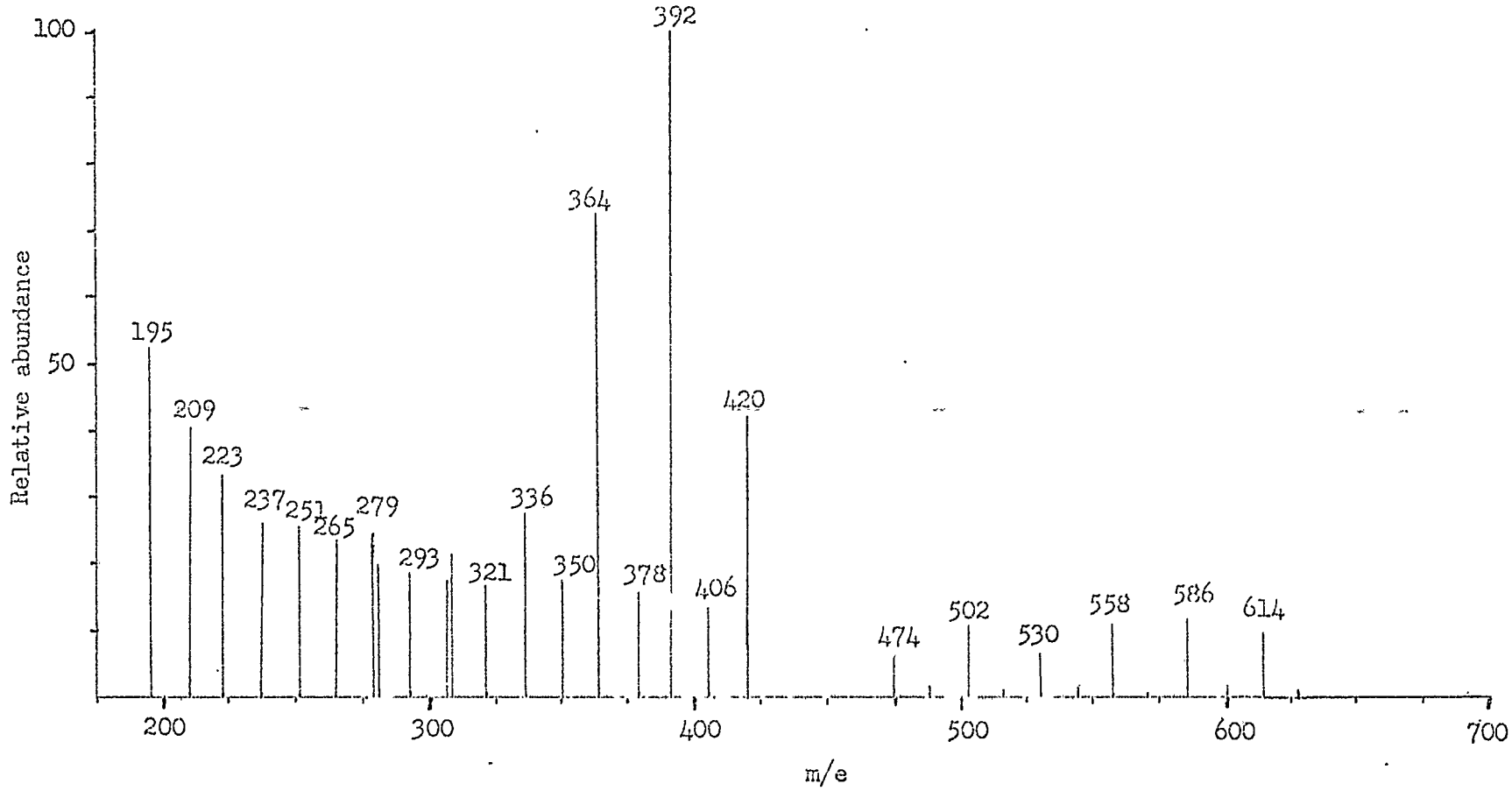
M^+	Formula	Relative* abundance
474	$C_{32}H_{58}O_2$	5.80
488	$C_{33}H_{60}O_2$	1.74
502	$C_{34}H_{62}O_2$	10.40
516	$C_{35}H_{64}O_2$	1.16
530	$C_{36}H_{66}O_2$	6.47
544	$C_{37}H_{68}O_2$	1.16
558	$C_{38}H_{70}O_2$	11.00
572	$C_{39}H_{72}O_2$	0.93
586	$C_{40}H_{74}O_2$	11.60
600	$C_{41}H_{76}O_2$	1.51
614	$C_{42}H_{78}O_2$	9.85
628	$C_{43}H_{80}O_2$	1.16

* Relative to peak at m/e 392 as 100.

Again one would expect a similar mechanism of fragmentation to that discussed previously. However, with the presence of unsaturation, there may be some additional fragments not present in the "saturated" fragmentation mechanism. Examination of the spectrum showed that the alcohol fragments (even mass numbers) were completely saturated. Examination of the acid fragments (odd mass numbers) confirmed immediately that the unsaturation

Figure 4

Portion of mass spectrum of Fraction F



was present in the acid fragments.

The alcohol fragments were first examined. The major fragments due to $\left[C_mH_{2m}\right]^+$ are listed in Table 8.

TABLE 8

Alcohol fragments of fraction F

$\left[C_mH_{2m}\right]^+$

M^+	m	Relative abundance
280	20	20.3
308	22	21.7
336	24	27.5
350	25	17.3
364	26	72.5
378	27	15.8
392	28	100.00
406	29	13.1
420	30	42.1

Fragments with mass numbers of general structure $\left[CH_3(CH_2)_m-O-\underset{\underset{OH}{|}}{C}=CH_2\right]^+$ were also found. No fragments of any appreciable intensity were found for acids corresponding to $\left[CH_3(CH_2)_nCO_2H_2\right]^+$ as would be expected if the acid fragment was saturated. However quite intense peaks were observed for triply unsaturated acid fragments. It appeared, therefore, that the acid components of F were tri-unsaturated. Mass numbers corresponding to the following unsaturated acid fragments were present (Table 9).

TABLE 9

Acid fragments of fraction F

$[CH_3C_nH_{2n-6}CO_2H_2]^+$

M^+	n	Relative abundance
195	10	52.3
209	11	40.6
223	12	33.4
237	13	26.1
251	14	25.7
265	15	23.2
279	16	24.6
293	17	18.8
307	18	17.5
321	19	16.5

Further examination of the mass spectrum showed that fragments with mass numbers corresponding to the ions $\left[CH_3C_nH_{2n-6}-\overset{O}{\overset{\|}{C}}\right]^+$ and $\left[CH_3C_nH_{2n-6}-\overset{O}{\overset{\|}{C}}-O-(CH_2)_2\right]^+$ were present.

By combining the acid and alcohol fragments to fit the mass numbers of the parent esters, the following table of proposed esters was formulated (Table 10).

TABLE 10

Possible formulae corresponding to parent peaks of esters in Fraction F

m/e 474	m/e 488
$C_{11}H_{17}CO_2H_{41}C_{20}$	$C_{12}H_{19}CO_2H_{41}C_{20}$
m/e 502	**m/e 516**
$C_{13}H_{21}CO_2H_{41}C_{20}$	$C_{14}H_{23}CO_2H_{41}C_{20}$
$C_{11}H_{17}CO_2H_{45}C_{22}$	$C_{12}H_{19}CO_2H_{45}C_{22}$
m/e 530	**m/e 544**
$C_{15}H_{25}CO_2H_{41}C_{20}$	$C_{16}H_{27}CO_2H_{41}C_{20}$
$C_{13}H_{21}CO_2H_{45}C_{22}$	$C_{14}H_{23}CO_2H_{45}C_{22}$
$C_{11}H_{17}CO_2H_{49}C_{24}$	$C_{12}H_{19}CO_2H_{49}C_{24}$
	$C_{11}H_{17}CO_2H_{51}C_{25}$
m/e 558	**m/e 572**
$C_{17}H_{29}CO_2H_{41}C_{20}$	$C_{18}H_{31}CO_2H_{41}C_{20}$
$C_{15}H_{25}CO_2H_{45}C_{22}$	$C_{16}H_{27}CO_2H_{45}C_{22}$
$C_{13}H_{21}CO_2H_{49}C_{24}$	$C_{14}H_{23}CO_2H_{49}C_{24}$
$C_{12}H_{19}CO_2H_{51}C_{25}$	$C_{13}H_{21}CO_2H_{51}C_{25}$
$C_{11}H_{17}CO_2H_{53}C_{26}$	$C_{12}H_{19}CO_2H_{53}C_{26}$
	$C_{11}H_{17}CO_2H_{55}C_{27}$
m/e 586	**m/e 600**
$C_{19}H_{33}CO_2H_{41}C_{20}$	$C_{20}H_{35}CO_2H_{41}C_{20}$
$C_{17}H_{29}CO_2H_{45}C_{22}$	$C_{18}H_{31}CO_2H_{45}C_{22}$
$C_{15}H_{25}CO_2H_{49}C_{24}$	$C_{16}H_{27}CO_2H_{49}C_{24}$
$C_{14}H_{23}CO_2H_{51}C_{25}$	$C_{15}H_{25}CO_2H_{51}C_{25}$
$C_{13}H_{21}CO_2H_{53}C_{26}$	$C_{14}H_{23}CO_2H_{53}C_{26}$

$C_{12}H_{19}CO_2H_{55}C_{27}$	$C_{13}H_{21}CO_2H_{55}C_{27}$
$C_{11}H_{17}CO_2H_{57}C_{28}$	$C_{12}H_{19}CO_2H_{57}C_{28}$
	$C_{11}H_{17}CO_2H_{59}C_{29}$

m/e 614	m/e 628
$C_{19}H_{33}CO_2H_{45}C_{22}$	$C_{20}H_{35}CO_2H_{45}C_{22}$
$C_{17}H_{29}CO_2H_{49}C_{24}$	$C_{18}H_{31}CO_2H_{49}C_{24}$
$C_{16}H_{27}CO_2H_{51}C_{25}$	$C_{17}H_{29}CO_2H_{51}C_{25}$
$C_{15}H_{25}CO_2H_{53}C_{26}$	$C_{16}H_{27}CO_2H_{53}C_{26}$
$C_{14}H_{23}CO_2H_{55}C_{27}$	$C_{15}H_{25}CO_2H_{55}C_{27}$
$C_{13}H_{21}CO_2H_{57}C_{28}$	$C_{14}H_{23}CO_2H_{57}C_{28}$
$C_{12}H_{19}CO_2H_{59}C_{29}$	$C_{13}H_{21}CO_2H_{59}C_{29}$
$C_{11}H_{17}CO_2H_{61}C_{30}$	$C_{12}H_{19}CO_2H_{61}C_{30}$

The unsaturated fraction (F) was reduced with hydrogen in the presence of Adam's catalyst. The carbonyl absorption peak shifted from 1700 cm^{-1} to 1735 cm^{-1}. The three peaks originally observed at 1500, 1585 and 1625 cm^{-1} in F disappeared upon hydrogenation. The spectrum of the reduced compound was similar to that of the saturated aliphatic esters studied previously (Fraction E). The saturated esters obtained by reduction were hydrolyzed using alcoholic sodium hydroxide, the acids and alcohols which resulted were isolated in the usual manner and the acid fraction was methylated. The alcohols and methyl esters were then gas-chromatographed and the saturated normal alcohols (C_{22}, C_{24}, C_{26}, C_{28}, C_{30}) were identified as were the homologous saturated acids (C_{13} - C_{20}) as their methyl esters. The presence of the C_{13} acid ester was not definitely established as the peak corresponding to this ester was poorly resolved from the solvent peak; it did have the correct retention time for a thirteen carbon acid. All resolution was lost

when the temperature was lowered. Taking the results obtained from gas-chromatography into consideration it was possible to decide which of the proposed esters (Table 10) may be present in fraction (F).

The unsaturated ester fraction (F) was also hydrolyzed with alcoholic potassium hydroxide. The saturated alcohols (C_{22}, C_{24}, C_{26}, C_{28}, C_{30}) were isolated and identified by means of GLC. The results obtained agreed with those deduced from the mass spectrum showing that the alcohols were in fact saturated in the original material. The unsaturated acids were isolated as liquids but they were not methylated and identified as their methyl esters as no standard triply unsaturated acids other than linolenic acid were available for the preparation of a standard curve. This investigation confirmed, however, that the unsaturation is located in the acid fragment of the esters (F) as proposed.

Once again the presence of both the alcohol and acid fragment in the gas-chromatographic analysis was used as a basis to decide which of the proposed esters were most probably present. The results are illustrated in Table 11.

TABLE 11

The probable component esters of fraction F.

m/e 474	m/e 488
* $C_{11}H_{17}CO_2H_{41}C_{20}$	* $C_{12}H_{19}CO_2H_{41}C_{20}$
m/e 502	**m/e 516**
* $C_{13}H_{21}CO_2H_{41}C_{20}$	$C_{12}H_{19}CO_2H_{45}C_{22}$
m/e 530	**m/e 544**
$C_{13}H_{21}CO_2H_{45}C_{22}$	$C_{14}H_{23}CO_2H_{45}C_{22}$
	$C_{12}H_{19}CO_2H_{49}C_{24}$

m/e 558	m/e 572
$C_{15}H_{25}CO_2H_{45}C_{22}$	$C_{16}H_{27}CO_2H_{45}C_{22}$
$C_{13}H_{21}CO_2H_{49}C_{24}$	$C_{14}H_{23}CO_2H_{49}C_{24}$
	$C_{12}H_{19}CO_2H_{53}C_{26}$

m/e 586	m/e 600
$C_{17}H_{29}CO_2H_{45}C_{22}$	$C_{18}H_{31}CO_2H_{45}C_{22}$
$C_{15}H_{25}CO_2H_{49}C_{24}$	$C_{16}H_{27}CO_2H_{49}C_{24}$
$C_{13}H_{21}CO_2H_{53}C_{26}$	$C_{14}H_{23}CO_2H_{53}C_{26}$
	$C_{12}H_{19}CO_2H_{57}C_{28}$

m/e 614	m/e 628
$C_{19}H_{33}CO_2H_{45}C_{22}$	$C_{18}H_{31}CO_2H_{49}C_{24}$
$C_{17}H_{29}CO_2H_{49}C_{24}$	$C_{16}H_{27}CO_2H_{53}C_{26}$
$C_{15}H_{25}CO_2H_{53}C_{26}$	$C_{14}H_{23}CO_2H_{57}C_{28}$
$C_{13}H_{21}CO_2H_{57}C_{28}$	$C_{12}H_{19}CO_2H_{61}C_{30}$

* The starred (*) esters were not unambiguously identified as both the acid and alcohol fragments were not detected in the gas-chromatographic analysis.

No information was derived from the mass spectrum concerning the position of the double bonds. Long chain esters with one double bond in a position higher than six or seven from one end of the chain are not distinguishable from similar compounds irrespective of position and geometrical configuration of the double bond (95). For this reason, no deductions could be made regarding the positions of any of the three double bonds in the esters listed in Table 11.

Arcoleo and coworkers (72) isolated a mixture of free fatty acids,

(i.e. lauric, myristic, palmitic, stearic and oleic acids), as well as a mixture of esters of myristic, palmitic, stearic and oleic acids from the petroleum ether extracts of Opuntia ficus indica. The esters found in this study were not extracted in the defatting step as long chain esters are practically insoluble in cold petroleum ether. This is the only evidence of work with any similarity to the present studies.

The phenolic material (D) obtained after concentration of the ethanol mother liquor from the cactus extraction was hydrolyzed with aqueous sodium hydroxide. The free phenols obtained were methylated using diazomethane to improve their properties for gas-liquid chromatographic analysis (90).

The methylated phenolic material was chromatographed on a column of Silicone 550 Oil on acid-washed firebrick at 245°. The unknown sample was resolved into seven components under these conditions. Each of the seven components was collected directly onto potassium bromide (91) and an infrared spectrum obtained of each component. Examination of these infrared spectra showed that peaks 1 and 3 were due to mixtures of a saturated methyl ester and a phenolic compound, which was suggested by the double carbonyl absorption at 1735 cm^{-1} due to an aliphatic ester and 1715 cm^{-1} due to an aromatic ester. In addition, in both cases, there was strong absorption at 2920 and 2860 cm^{-1} which is characteristic of aliphatic compounds, while, on the other hand, there were strong bands at 1600 and 1500 cm^{-1} characteristic of aromaticity. Gas-chromatography of the methylated phenolic material was repeated at a lower temperature (215°). This time the unknown mixture was resolved into nine components. Peaks 1 and 3 in the first separation (245°) were each separated into two components under these conditions. Again each component was collected directly onto potassium bromide and an infrared

spectrum obtained for each of the nine components.

A comparison of these infrared spectra with those of authentic samples prepared earlier for the Poplar studies, and a comparison of the retention times of these unknowns with the retention times of the authentic samples as well as reference to the standard curve of methyl esters prepared by plotting the log of the retention time versus the number of carbon atoms in the methyl ester permitted the identification of most of the components. (Table 12).

TABLE 12

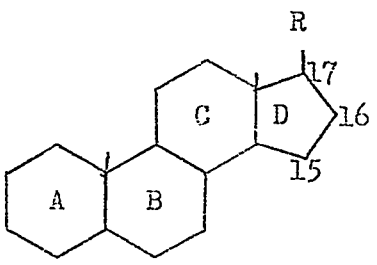
Acidic components of hydrolyzed acid fraction (D)

Fraction	Retention time (min.) of methyl deriv.	% of total	Identity of methyl deriv.
1	5.3	1.47	methyl p-methoxybenzoate
2	6.25	1.78	methyl undecylate
3	8.2	1.83	methyl laurate
4	11.6	3.92	methyl 3,4-dimethoxybenzoate
5	12.48	4.14	methyl myristate
6	17.8	1.81	methyl pentadecylate
7	26.4	21.47	methyl palmitate
8	35.4	6.10	methyl 3,4-dimethoxycinnamate
9	54.5	57.45	methyl stearate

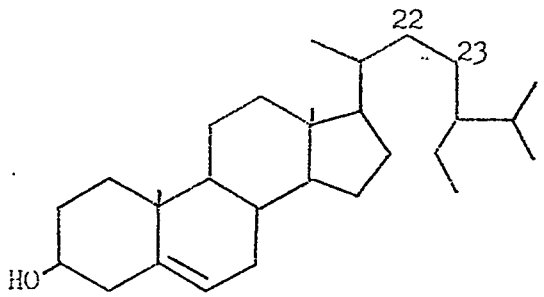
Isolation of both phenolic and aliphatic acids after hydrolysis suggests that the phenolic material (D) was probably a mixture of phenolic esters or glucosides.

The present work appears to be the first report of the presence of phenolic material in cacti. It also appears to be one of the first times

that β-sitosterol was not isolated from an Opuntia species. In previous work on other species of cacti, β-sitosterol has usually been found in quite significant amounts. Had β-sitosterol been present, it should have appeared in the unsaturated fraction (F) obtained by column chromatography of (G). β-sitosterol has previously been eluted quite easily from alumina columns (19). The other fractions were saturated and so would not contain any β-sitosterol.



(A)



β-sitosterol

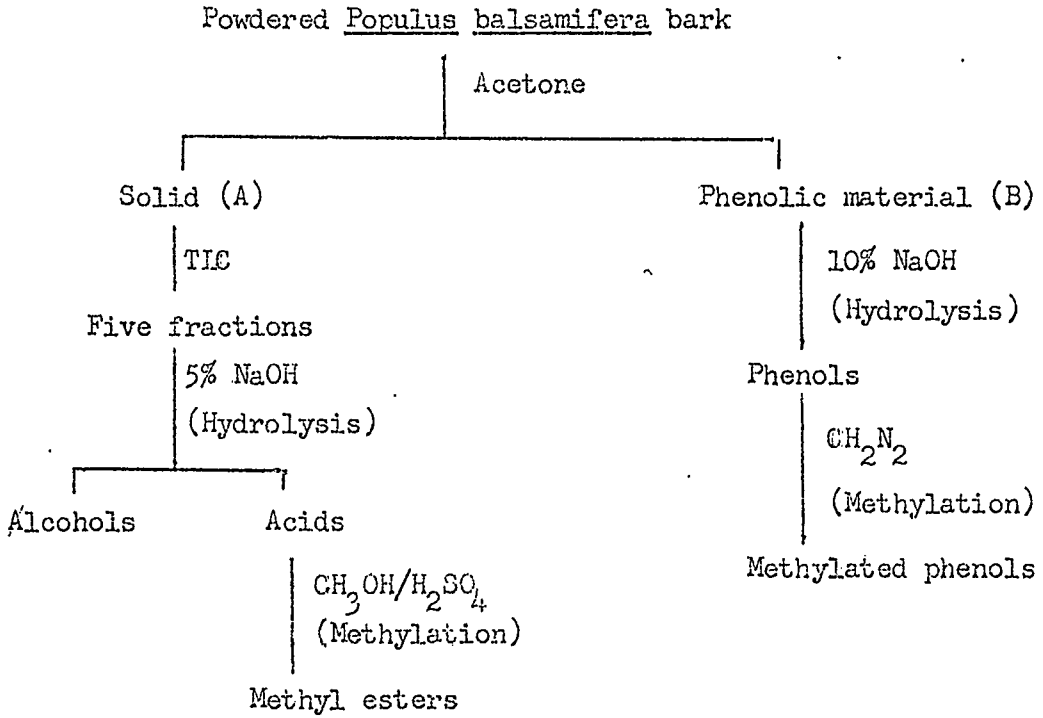
The major fragmentation processes (95) of steroids in the mass spectrometer involve cleavage of the side chain; loss of an angular methyl group; loss of the elements of water if hydroxyl or keto groups are present and fragmentation of the ring system. Cleavage of the ring system is frequently accompanied by the rearrangement of a hydrogen atom. It is known that steroids of the general structure (A) above have a tendency to lose the R group either as such or, to an even greater extent, to lose the fragment R + 42. The most energetically reasonable origin of the 42 mass units lost along with the side chain is C-15, C-16 and C-17.

No peak was observed in the mass spectrum at m/e 414 which would correspond to β- sitosterol. There appeared a peak of very weak intensity at m/e 399 which could result from the loss of an angular methyl (M-15). Similarly a very weak peak at m/e 396 (m-18) was present which could result

from the loss of water. However there were no peaks at m/e 273 (m-141) which would result from cleavage of the side chain or at m/e 231 (m-(R + 42)) which would result from cleavage of the side chain along with fragmentation of ring D to lose C-15, C-16 and C-17 accompanied by rearrangement of a hydrogen atom. This evidence supports the assumption that β -sitosterol was not present in Opuntia fragilis.

SCHEMATIC II

Schematic of Extraction and Isolation Procedure



IV EXPERIMENTAL

(A) Populus balsamifera

The Populus balsamifera bark was supplied by F. H. Hewett of the Forestry Branch, Department of Natural Resources, Prince Albert, Saskatchewan. Gas-liquid chromatography was performed on an "Aerograph A-700" instrument. Melting points are uncorrected. They were determined on a Fisher-Johns melting point apparatus using the flat plate method. The infrared spectra were determined on a Beckman IR-8 or a Unicam SP-200 spectrometer. Analysis were performed on an F and M Model "185" C, H and N Analyzer. The mass spectra were recorded on a MS-9 mass spectrometer using the direct probe method and were kindly determined for us at the University of Alberta, Edmonton.

4.1.1 Preparation of the Bark

The dried Populus balsamifera bark, freed of any heartwood, was powdered using a Fitz mill equipped with a No. 20 screen and cutting knives. The powdered bark was used as such for subsequent extractions.

4.1.2 Extraction of the Powdered Bark

The powdered bark (1120 g.) from the Populus balsamifera tree was extracted continuously (6 hrs) in a Soxhlet extractor using acetone (6 liters). The acetone was removed, another six liters of fresh acetone were added, and the extraction continued for an additional sixteen hours. Upon cooling, a white solid (A) (34.25 g.), (m.p. 78-79°), which gradually darkened on exposure to air, settled out from the combined acetone extractions and was removed by filtration. The acetone from the mother liquor was removed by distillation leaving a dark brown solid (B; 10.5 g.), which gave a purple color when treated with ferric chloride. B was soluble in 5%

aqueous sodium hydroxide but was insoluble in 5% aqueous sodium bicarbonate.

4.1.3 Preparative Thin Layer Chromatography of A

A portion of A (8.38 g.) was chromatographed on 152 Silica gel G plates (20 x 20 cm.), 1.3 mm. in thickness, using benzene: dioxan: glacial acetic acid (90:25:4) as the developing solvent. Under these conditions A was separated into five fractions which fluoresced blue under ultraviolet light. Each of the five fractions was scraped off the plates separately. The silica gel fractions were each extracted continuously (24 hrs) with acetone (125 ml) in a Soxhlet extractor. The acetone was removed by aerial evaporation. The following results were obtained (Table 13).

TABLE 13

Fractions from Preparative Thin Layer Chromatography of A

Fraction	R_f	Weight (g)	m.p.
1	0.64	0.7267	76-78°
2	0.71	0.4870	72-74°
3	0.76	0.9806	71-73°
4	0.81	0.3190	68-69°
5	0.98	0.1246	67-68°

The remainder of the material (coloring matter and debris) failed to migrate from the origin.

Rechromatography, under the same conditions as above, of a very small aliquot of each of these five fractions showed that only fraction 5 apparently consisted of a single component. Fractions 1-4 were mixtures. Fractions 1, 2 and 3 each gave rise to three spots while fraction 4 gave rise to two spots.

4.1.4 Hydrolysis of A and Methylation of the Acids

A portion of neutral material A (2.01 g.) was hydrolyzed with 5% w/v aqueous sodium hydroxide (125 ml) for four hours. The alcohols which resulted were extracted into ether (200 ml.) and the product obtained on removal of the ether was defatted with light petroleum (b.p. 60-68°) (5 ml), then recrystallized from ethanol. In this way a product (0.447 g.), m.p. 82-83°, was obtained. The hydrolysis mixture was acidified and extracted with ether (200 ml.). A mixture of acids (0.728 g.), m.p. 78-80°, was obtained after crystallization from ethanol of the product remaining on removal of the ether.

A portion (0.6153 g.) of the mixture of acids was heated under reflux (20 hrs) with concentrated sulfuric acid (0.1 ml) in methanol (20 ml). On cooling the reaction mixture a brownish white solid separated. Water (25 ml) was added and the precipitated methyl esters were extracted with ether (50 ml). The ether solution was washed with 2% aqueous sodium hydroxide (3 x 15 ml), and then with water (3 x 30 ml). The ethereal layer was dried ($CaCl_2$) and evaporated to give the methyl esters which, on recrystallization from ethanol, gave the methyl esters (0.447 g; 72.5% yield), m.p. 68-70°.

4.1.5 Preparation of the Standard Curves

The standard curve for the alcohols (Figure 1) was obtained by plotting log retention time versus chain length using the C_{24}, C_{26} and C_{28} normal aliphatic alcohols. The alcohols were chromatographed under the following conditions:

Column: 0.3% SE-30 on glass beads (100/120 mesh) (5' x $\frac{1}{4}$").

Column temperature: 245°

Helium flow rate: 200 ml/minute

Inlet pressure: 35 p.s.i.

The standard curve (Figure 2) for the methyl esters was obtained by plotting log retention time *versus* chain length using palmitic, stearic, arachidic, behenic, lignoceric, cerotic, palmitoleic, oleic, linoleic, linolenic, arachidonic and erucic acid methyl esters. The methyl esters were chromatographed under the following conditions:

Column: 16% w/w butanediol succinate on acid-washed Celite 545 ($2.5' \times \frac{1}{4}''$)

Column temperature: 220°

Helium flow rate: 60 ml/minute

4.1.6 *Identification of the normal aliphatic alcohols obtained by saponification of neutral material A.*

The five alcohol fractions obtained from the preparative thin layer chromatography (section 4.1.3) followed by hydrolysis (see 4.1.4) were each subjected to gas chromatography under the same conditions as were the standard alcohols (see 4.1.5); the log retention time was calculated for each component and the chain length of the alcohol was deduced from the standard curve. The following results were obtained (Table 14).

TABLE 14

Normal aliphatic alcohols present in esters (A)

R_f of ester fraction	n in $C_nH_{2n+1}OH$	% of total alcohols in fraction
0.62	20	0.63
	22	5.20
	24	7.68
	25	8.86
	26	47.90
	28	29.60

TABLE 14 (continued)

R_f of ester fraction	n in $C_nH_{2n+1}OH$	% of total alcohols in fraction
0.71	20	1.59
	22	1.59
	24	6.82
	26	47.70
	28	42.40
0.76	20	1.40
	22	1.40
	24	4.70
	25	1.47
	26	49.80
	28	41.30
0.81	18	0.17
	20	0.54
	22	2.97
	24	8.10
	25	2.85
	26	45.70
	28	39.70
0.98	18	0.63
	20	4.42
	22	8.94
	24	7.31
	25	1.62
	26	43.0
	28	34.0

4.1.7 Identification of the acid components (as their methyl esters) obtained by saponification of neutral material A

The mixture of methyl esters obtained as described in section 4.1.4, was gas-chromatographed under the same conditions as was used for the reference standards (see section 4.1.5). The log retention time was calculated for the unknown methyl esters from each of the five fractions. The following results were obtained (Table 15).

TABLE 15

Aliphatic acids present in esters (A)

R_f of ester fraction	Acid	% of total acids in fraction
0.62	Palmitic (C_{16})	0.57
	Palmitoleic (C_{16})	0.17
	Oleic (C_{18})	0.28
	11-Eicosenoic (C_{20})	1.17
	Behenic (C_{22})	13.5
	Lignoceric (C_{24})	42.4
	Cerotic (C_{26})	41.8
0.71	Palmitic	1.13
	Palmitoleic	0.57
	Behenic	10.50
	Lignoceric	47.30
	Cerotic	40.50

TABLE 15 (continued)

R_f of ester fraction	Acid	% of total acids in fraction
0.76	Palmitic	1.49
	Palmitoleic	0.33
	Oleic	0.65
	Behenic	13.0
	Lignoceric	46.3
	C_{24} unsaturated, or C_{25}*	2.92
	Cerotic	35.3
0.81	Palmitic	16.4
	Palmitoleic	6.84
	Stearic	8.20
	Linolenic (C_{18})	3.90
	Behenic	21.80
	Lignoceric	42.90
0.98	Palmitic	9.66
	Palmitoleic	8.12
	Stearic	7.57
	Oleic	3.24
	Linolenic	5.17
	Behenic	21.20
	Lignoceric	31.40
	Cerotic	13.50

* No C_{24} unsaturated acid was available for comparison.

4.1.8 Preparation of Authentic Esters

The appropriate acid (20 mg.) was heated under reflux for 24 hours in benzene (30 ml.) with an excess of thionyl chloride. The excess thionyl chloride was removed by repeated addition and then distillation of benzene until the odour of thionyl chloride was no longer present. The alcohol (approx. 14 mg) was added and heating under reflux continued for a further 24 hours. The resulting mixture was extracted with 10% aqueous sodium hydroxide (2 x 10 ml), and then washed with water (2 x 10 ml). The benzene solution was dried ($CaCl_2$), and the benzene was removed by distillation. Small volumes of dry benzene were added repeatedly, then distilled off to remove any water still present as water of recrystallization. The ester obtained was recrystallized from light petroleum (b.p. 40 - 60°), and stored in a desiccator until analyzed. The following esters were prepared and analyzed (Table 16).

TABLE 16

Authentic esters prepared

Ester	Formula	m.p.	Analysis Calculated C	Calculated H	Found C	Found H
1. Stearyl stearate	$C_{36}H_{72}O_2$	61-62°	80.59	13.43	80.32	13.32
2. Stearyl arachidate	$C_{38}H_{76}O_2$	67-68°	80.78	13.56	80.30	13.50
3. Stearyl behenate	$C_{40}H_{80}O_2$	71-72°	81.00	13.60	81.31	13.54
4. Cerotyl behenate	$C_{48}H_{96}O_2$	80-81°	81.74	13.72	81.82	13.50
5. Octacosanyl behenate	$C_{50}H_{100}O_2$	82-83°	81.89	13.75	81.62	13.50
6. Octacosanyl lignocerate	$C_{52}H_{104}O_2$	84-85°	82.03	13.77	81.82	13.50

4.1.9 Thin Layer Chromatography of Authentic Esters

These esters were chromatographed under the same conditions as the unknown esters (as in 4.1.3). The following results were obtained (Table 17).

TABLE 17

T.L.C. of Long-chain fatty esters

Ester	Total no. of C atoms	R_f Run 1	Run 2	Run 3
Stearyl stearate	36	0.96	0.93	0.94
Stearyl arachidate	38	0.95	-	-
Stearyl behenate	40	0.92	0.90	0.93
Cerotyl behenate	48	0.90	0.88	0.91
Octacosanyl behenate	50	-	0.83	-
Octacosanyl lignocerate	52	-	-	0.87

Cetyl behenate was also synthetized but not analyzed. Cetyl behenate and stearyl arachidate were chromatographed under the same conditions as previously described, (section 4.1.3). The results are given in Table 18.

TABLE 18

T.L.C. of stearyl arachidate and cetyl behenate

	R_f Stearyl arachidate	R_f Cetyl behenate	R_f Mixture
(large sample)	0.83	0.85	0.84
	0.90	0.91	0.92
(small sample)	0.965	0.965	0.97

4.2.1 Hydrolysis of Acidic Material B

A portion of acidic material B (30 g.) obtained as described in section 4.1.2 was heated under reflux with 10% aqueous sodium hydroxide (125 ml) for five hours. The cooled mixture was extracted with ether (200 ml) and the ethereal extract discarded. The aqueous layer was acidified with concentrated sulfuric acid and extracted with ether (200 ml). The ether was evaporated under reduced pressure to give phenolic material (14 g) as a brown viscous oil.

4.2.2 Methylation of Phenolic Material from B.

The above phenolic material (14 g) was dissolved in dry ether (200 ml) and a solution of diazomethane (from 34 g. of N-nitrosomethylurea) in ether was added in three aliquots at 3 hour intervals. Effervescence was quite vigorous at first but gradually slowed down after the first methylation. After the third methylation a brown powdery organic solid (0.49 g), m.p.> 230° which was insoluble in ether and gave a negative ferric chloride test was filtered off and rejected. An ether solution of the methylated phenolic

material was extracted with 10% aqueous sodium hydroxide (2 x 100 ml). Acidification of the basic aqueous layer and extraction with ether gave a black tar (0.9 g) which gave a purple color with ferric chloride. The original ether layer after extraction with aqueous sodium hydroxide was washed with water, dried ($CaCl_2$) and then concentrated to a volume of approximately 10 ml. The resulting brown viscous liquid was used as such for gas-chromatographic analysis.

Preparation of diazomethane (97)

Nitrosomethylurea was prepared by treating acetamide (59 g) with bromine (88 g), followed by 25% aqueous sodium hydroxide (160 ml) which gave acetylmethylurea (50 g). The acetylmethylurea was treated with concentrated hydrochloric acid (50 ml) to give methylurea which, when treated with sodium nitrite (38 g), gave nitrosomethylurea (34 g), m.p. 123-124°.

Treatment of nitrosomethylurea (34 g) in ether (200 ml) with 50% aqueous potassium hydroxide solution (60 ml) produced diazomethane which was distilled at 50° and collected into ether.

4.2.3 Gas-chromatography of Methylated Reference Phenols

Several phenolic acids, aldehydes and ketones (Table 19) were each dissolved in ether and methylated using an excess of an ethereal solution of diazomethane. The ether solution was then extracted with 10% aqueous sodium hydroxide, washed with water, dried ($CaCl_2$) and gas-chromatographed under the following conditions:

Column: 25% Silicone 550 Oil on Acid-washed firebrick (40-60 mesh) (6' x $\frac{1}{4}$").

Temperature: 245°

Helium flow rate: 80 ml/minute.

Each one of the standards was collected directly onto potassium bromide (approx. 50 mg) as the compound came out the collector tip. An infrared spectrum was recorded for each one of the methylated standards. The following standards were chromatographed (Table 19).

TABLE 19

Retention Times of Methylated Reference Compounds

Identity of Reference Compound	Retention time of Methyl deriv. (min)
Phenol	0.65
p-Hydroxybenzaldehyde	1.95
p-Hydroxybenzoic acid	2.6
Cinnamic acid	3.0
Vanillin	3.65
Acetovanillone	4.65
Vanillic acid	4.95
Syringaldehyde	5.55
p-Coumaric acid	6.8
Acetosyringone	6.85
Syringic acid	7.7
Ferulic acid	13.1

4.2.4 Chromatographic Analysis of the Unknown Methylated Phenols

A portion of the methylated unknown sample (section 4.2.2) was gas-chromatographed under the same conditions as those used for the standards (section 4.2.3). The unknown mixture was resolved into thirteen components, each of which was collected directly onto potassium bromide (approx. 50 mg). An infrared spectrum was recorded for each of the thirteen components. The results are illustrated in Table 1.

The infrared spectra of Fractions 1, 2, 3, 5, 10, 12 and 13 (KBr discs) exhibited bands at 2920 (s), 2860 (s), 1735 (s), 1460 (s), 1377 (w), 1260 (m), 1170 (m), 724 (w) and 714 (w) cm^{-1}.

Fraction 4: This fraction exhibited bands in the infrared (KBr disc) at 2920 (w), 1715 (s), 1605 (s), 1510 (s), 1430 (s), 1270 (s), 1100 (s), 1030 (s), 965 (m), 800 (s), 770 (s) and 690 (s) cm^{-1}. A direct comparison with authentic methyl p-methoxybenzoate showed the two compounds to be identical in all respects.

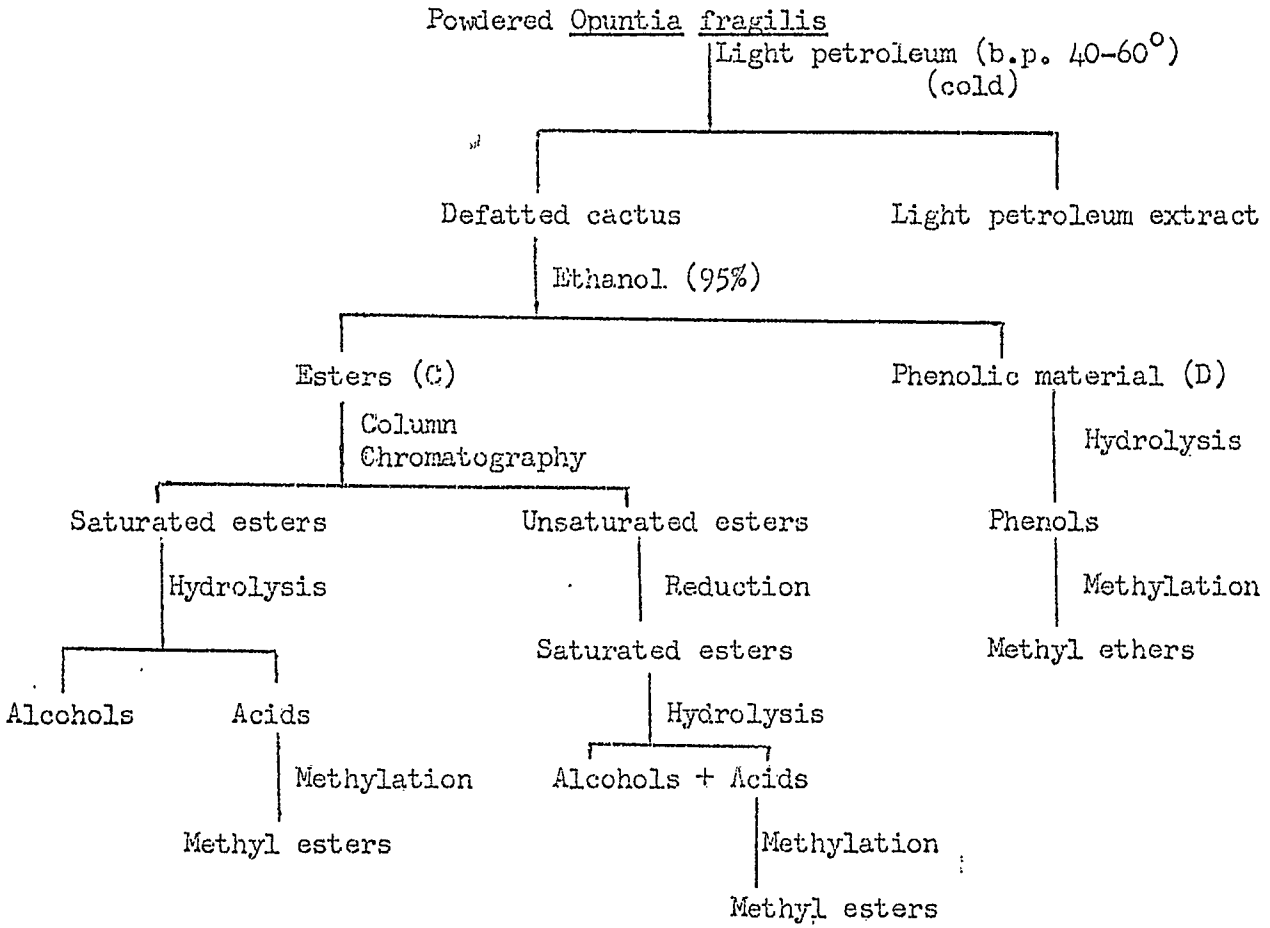
Fraction 7: It exhibited the following bands in the infrared (KBr disc): 2940 (w), 1715 (s), 1600 (m), 1515 (m), 1465 (m), 1430 (m), 1415 (m), 1300 (s), 1275 (s), 1230 (m), 1190 (w), 1170 (w), 1240 (m), 1210 (m), 1120 (m), 1090 (w), 870 and 760 (s) cm^{-1}. These data are identical with those for authentic methyl 3,4-dimethoxybenzoate.

Fraction 8: It had infrared absorption bands (KBr disc) at 2940 (w), 1715 (s), 1535 (w), 1600 (m), 1510 (s), 1430 (w), 1288 (m), 1255 (m), 1205 (m), 1175 (s), 1025 (w), 980 (M) (trans CH=CH), 840 (w) and 820 (w) cm^{-1}. The infrared spectrum was identical in all respects with that of authentic methyl p-methoxycinnamate.

Fraction 9: The infrared exhibited bands at 2940 (w), 1715 (s), 1600 (s), 1510 (s), 1460 (w), 1430 (m), 1255 (m), 1165 (s), 1030 (m) and 780 (m) cm^{-1}. The identity of this compound was not established.

Fraction 11: This compound exhibited bands in the infrared at 2940 (w), 1695 (s), 1620 (w), 1595 (s), 1510 (s), 1460 (m), 1435 (m), 1420 (w), 1250 (s), 1160 (m), 1140 (m), 1038 (m), 1020 (m), 980 (m) (trans CH=CH), 870 (w) and 815 (m) cm^{-1}. A direct comparison with authentic methyl 3,4-dimethoxycinnamate showed the two compounds to be identical in all respects.

SCHEMATIC III



(B) Opuntia fragilis

The cactus was collected (October 21, 1965) at Pike Lake, near Saskatoon and identified as Opuntia fragilis by Dr. B. J. Sallons, Canada Department of Agriculture, Research Station, University of Saskatchewan, Saskatoon.

4.3.1 Extraction Procedure

The cactus was ground in a Fitz mill equipped with a No. 20 screen. The resulting wet green granular solid was dried in air (48 hrs). A portion of the dried powdered cactus (200 g) was defatted by suspending it in light petroleum (b.p. 40-60°) (400 ml) for 3 hours. Concentration of the light petroleum extract yielded a dark brown oily substance (0.4 g). The defatted cactus (200 g) was extracted in a Soxhlet extractor for 8 hours with 95% ethanol (1 l). The solvent was replaced with fresh ethanol (1 l) and the extraction continued for another 15 hours. Upon cooling, a white solid (C) (1.18 g), m.p. 85°, settled out from the first extraction and a further 224 mg of the same white solid, m.p. 85-88°, precipitated from the second extraction. The combined filtrates were evaporated under reduced pressure to give a dark green semi-solid (D) (ca 10 g.).

The marc from the ethanol extraction was basified with ammonium hydroxide and extracted for 20 hours with chloroform (1 l) in a Soxhlet extractor. A solid separated on concentration of the chloroform extract and it was recrystallized from benzene to give pale brown needles (30 mg), m.p. 75-76°. It exhibited bands in the infrared (KBr disc) at 3340 (s), 3180 (s), 2920 (w), 2860 (w), 1670 (s), 1390 (m), 1140 (m) and 700 (s) cm^{-1}. A direct comparison with acetamide showed the two compounds to be identical in all respects.

4.3.2 Column Chromatography of C

A portion of C (1.5 g) was dissolved in carbon tetrachloride (40 ml) and mixed with Type H adsorption alumina (2 g). The carbon tetrachloride was removed in a film evaporator and the coated alumina was placed on a column (30 cm) of alumina which was eluted as follows:

1) Light petroleum (b.p. 40-60°): A white waxy solid (98.2 mg), m.p. 73-74° was obtained.

2) Light petroleum : benzene (3 : 1)
3) Light petroleum : benzene (1 : 1)
4) Light petroleum : benzene (1 : 3)

} Fraction E

The combined fractions gave a white solid (205 mg), m.p. 77-78°.

5) Benzene: A white solid (20 mg) was obtained.

6) Benzene: ether (1 : 1): A white solid (18 mg) was isolated.

7) Ether : ethanol (1 : 1): A white solid (41 mg), m.p. 82-83°, was obtained.

8) Ethanol (95%): A white solid (3 mg), m.p. 82-83°, was isolated.

9) Acetic acid (5%): Aluminum acetate was the only product isolated.

Another chromatography of C (1.074 g) was carried out on alkaline alumina, Brockman activity 1 (120-200 mesh). The column was eluted as follows:

1) Light petroleum (b.p. 40-60°): A white waxy solid (20 mg), m.p. 73-74°, was isolated.

2) Light petroleum : benzene (1 : 1): A white solid (16 mg), m.p. 80°, was obtained.

3) Benzene
4) Benzene : ether (1 : 1)

} A white solid (9 mg) was isolated.

5a) Ether : ethanol (1 : 1): A white solid (27 mg), m.p. 82°, with absorptions in the infrared (KBr disc) at 3400, 2920, 2860, 1460, 1050 (-OH), 724 and 714 cm^{-1} was obtained. This spectrum was typical of long chain saturated aliphatic alcohols isolated in previous work. These alcohols were subjected to gas-chromatographic analysis employing the conditions previously used (section 4.1.5). The results are summarized in Table 20.

TABLE 20

Alcohols from Fraction 5a

n in $C_nH_{2n+1}OH$	% of total alcohols in fraction
22	5.36
24	5.19
26	13.12
28	76.31

5b) Ether : ethanol (1 : 1): A white solid (25 mg), m.p. 82-83° was obtained.

6) Ethanol: A white solid (1 mg) was obtained.

4.3.3 Mass Spectroscopy of Esters

For the mass spectroscopic analysis the saturated aliphatic ester fraction (E), m.p. 77-78°, was recrystallized from benzene and the unsaturated aliphatic ester fraction (F) was recrystallized from benzene.

4.3.4 Hydrolysis of Fraction E

The saturated esters (E) (110 mg) were boiled under reflux with potassium hydroxide (8 g) in 95% ethanol (25 ml) and water (15 ml) for five

hours. The mixture was cooled, water (100 ml) was added, and the mixture extracted with ether (200 ml). The ether solution was dried ($CaCl_2$) and evaporated to yield a solid which was defatted with light petroleum (b.p. 60-68°) (4 ml). A mixture of alcohols (50 mg), m.p. 82°, was left behind. The aqueous solution remaining after the ether extraction was acidified with concentrated hydrochloric acid and extracted with ether (200 ml). The ether solution was dried ($CaCl_2$) and evaporated to give a mixture of acids (37 mg), which were dissolved in methanol (25 ml) containing concentrated sulfuric acid (0.125 ml). The solution was boiled under reflux for 3 hours and the methyl esters isolated as described earlier (section 4.1.4).

4.3.5 Identification of the alcohols and methyl esters

The alcohols and methyl esters obtained as described in section 4.3.4 were analyzed by gas-chromatography using the same conditions as were used in section 4.1.5. The results are summarized in Tables 21 and 22.

TABLE 21

Aliphatic alcohols obtained after hydrolysis of fraction E

n in $C_nH_{2n+1}OH$	% of total alcohols in fraction
20	3.37
22	2.62
24	1.90
26	12.93
28	74.06
30	5.10

TABLE 22

Aliphatic acids obtained after hydrolysis of fraction E

Acids (Identified and chromatographed in the form of their methyl esters)	% of total acids in fraction
Palmitic	25.61
Heptadecanoic	3.02
Stearic	26.53
Nonadecanoic	1.53
Arachidic	13.77
Heneicosanoic	8.86
Behenic	20.51

4.3.6 Reduction of fraction F and hydrolysis of the reduced products

A portion of the unsaturated esters (F) (31 mg) were hydrogenated for 8 hours in a medium pressure hydrogenator (42 p.s.i.) in the cold using ether : ethanol (1 : 1) (20 ml) as the solvent, in the presence of platinum oxide (8 mg) and one drop of concentrated hydrochloric acid. The catalyst was filtered off by filtering the solution first through filter paper, then through a fine sintered glass funnel. The solvent was evaporated to give the saturated esters (30 mg), m.p. 77°. The saturated esters (30 mg) were hydrolyzed as described in section 4.3.4. The acids and alcohols were isolated as before (section 4.1.4) and the acids methylated (section 4.1.4). The alcohols and methyl esters were analyzed by gas-chromatography using the conditions described in section 4.1.5. The results are summarized in Table 23.

TABLE 23

Aliphatic alcohols obtained after reduction and hydrolysis of fraction F.

n in $C_nH_{2n+1}OH$	% of total alcohols in fraction
22	1.98
24	5.94
26	7.92
28	64.36
30	19.80

The following aliphatic acids; n-tridecanoic, myristic, n-pentadecanoic, palmitic, n-heptadecanoic, stearic, n-nonadecanoic, and arachidic acids; were isolated after reduction and hydrolysis of fraction F. Their retention times in minutes were 1.1, 1.5, 2.0, 2.6, 3.3, 4.3, 5.9 and 7.7 respectively.

4.3.7 Hydrolysis of fraction F

A portion of the unsaturated esters (F) (30 mg) was boiled under reflux with potassium hydroxide (8 g), 95% ethanol (25 ml), and water (15 ml) for 8 hours. The alcohols were isolated as described in section 4.1.4. Acidification of the aqueous solution with concentrated hydrochloric acid followed by extraction with ether (200 ml), drying ($CaCl_2$), and evaporation of the ether gave one drop of unsaturated acids as a colorless liquid.

The alcohols were subjected to gas-chromatography (see section 4.1.5). The results are summarized in Table 24.

TABLE 24

Aliphatic alcohols obtained after hydrolysis of fraction F.

n in $C_nH_{2n+1}OH$	% of total alcohols in fraction
22	1.75
24	7.9
26	6.4
28	64.8
30	19.1

4.3.8 Hydrolysis of phenolic material D

A portion of the phenolic fraction D (10 g) was boiled under reflux with 10% aqueous sodium hydroxide (125 ml). The solution was cooled, acidified with concentrated hydrochloric acid and extracted with ether (200 ml). The ethereal solution was dried ($CaCl_2$) and methylated using an excess of diazomethane in ether. Diazomethane was added repeatedly over a period of 4 hours. The solution was allowed to stand overnight and then extracted with 10% aqueous sodium hydroxide (50 ml). The ethereal layer was dried ($CaCl_2$) and the solvent evaporated down to a small volume (5 ml). The solution of the methyl ethers was used as such for gas-chromatographic analysis.

4.3.9 Gas-chromatography of the methylated phenols from D.

A portion of the methylated material was subjected to gas-chromatographic analysis using the following conditions:

Column: 25% Silicone 550 Oil on acid-washed firebrick (40-60 mesh) (6' x $\frac{1}{4}$").

Column temperature: 215°

Helium flow rate: 80 ml/minute.

Inlet pressure: 35 p.s.i.

The unknown mixture was resolved into 9 components under these conditions, each of which was collected directly onto potassium bromide (ca. 50 mg). An infrared spectrum was recorded for each of the 9 components. The results are summarized in Table 12.

The infrared spectra of fractions 2,3,5,6,7 and 9 (KBr discs) exhibited bands at 2920 (s), 2860 (s), 1735 (s), 1460 (s), 1377 (w), 1260 (m), 1170 (m), 724 (w) and 714 (w) cm^{-1}. Fraction 1 was methyl p-methoxybenzoate, fraction 4 was methyl 3,4-dimethoxybenzoate, and fraction 8 was methyl 3,4-dimethoxycinnamate. Their infrared spectra were identical with those of authentic samples. The infrared absorptions for these compounds are as given in section 4.2.4.

V. SUMMARY

(1) Acetone extraction of the bark of Populus balsamifera L. (Balsam poplar) gave neutral and acidic fractions. Saponification and gas-chromatographic analysis of the neutral material indicated that it was a mixture of long chain fatty esters, the acid components of which were palmitic, palmitoleic, stearic, oleic, linolenic, 11-eicosenoic, behenic, lignoceric, and cerotic acids; the component aliphatic alcohols were the straight chain ones with 18, 20, 22, 24, 25, 26, and 28 carbon atoms. Saponification of the acidic material, which consisted of phenols, phenolic esters and probably phenolic glycosides, yielded p-hydroxybenzoic, vanillic, p-coumaric and ferulic acids as well as caprylic, pelargonic, capric, tridecylic, palmitic, stearic and arachidic acids.

(2) Ethanol extraction of the Cactus, Opuntia fragilis. (Yellow cactus) gave neutral and acidic fractions. The neutral material was chromatographed on an alumina column from which a saturated and an unsaturated long chain aliphatic ester fraction were obtained. The saturated fraction was shown by mass spectroscopy to consist of a homologous series of esters with molecular weights 676, 690, 704, 718, 732, 746, 760, 774, 788 and 802, respectively. Similarly the unsaturated ester fraction was shown to consist of a homologous series of esters with molecular weights 474, 488, 502, 516, 530, 544, 558, 572, 586, 600, 614 and 628, respectively. The individual esters were identified by examination of the fragment ions in conjunction with gas-chromatographic analyses of the acid and alcohol components after saponification.

Saponification of the acidic material followed by methylation and

then gas-chromatographic analysis of the methylated products indicated the presence of p-hydroxybenzoic acid, vanillic acid and ferulic acid in addition to undecylic, lauric, myristic, pentadecylic, palmitic and stearic acids in this fraction.

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