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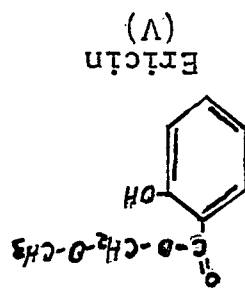
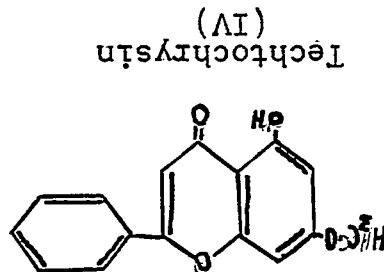
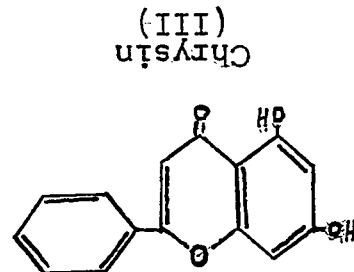
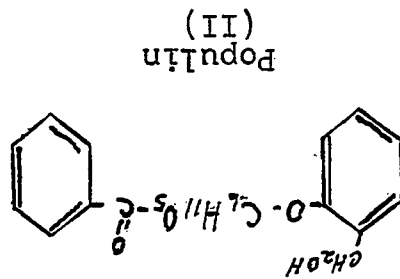
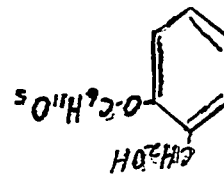
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SOME HEARTWOOD EXTRACTIVES OF *POPULUS TREMULOIDES*

I. INTRODUCTION

1. General

The study of the genus *Populus* is thought to have begun with Braconnot's isolation of the glycosides salicin (I) and populin (II) from the bark of *Populus alba* (1). Later workers showed these glycosides to be present in nine other species of poplar, along with a number of other common plant constituents such as chrysin (III), techtochrysin (IV), and ericin (V) (2).



In 1940, Sharikov and Kalina isolated the oil of the bark of Populus tremula and examined its physical properties. Thirteen years later, Wienhaus (6) examined the benzene extractives of aspen wood and the methanol extractives of wood previously extracted with benzene. In addition to examining the physical properties of the extractives, Wienhaus fractionated the extractives into free fatty acids, combined acids, and non-saponifiable matter. Little of his attention seems to have been directed towards the isolation and identification of individual compounds, and glucose and glycerol were the only compounds identified as

2. Populus tremula L.

species of aspen is summarized below.

The work carried out on the extractives from the two but not with the chemistry of the extraneous compounds. hydrolysis of the lignin or lignans present in the wood, papers by Pearl and co-workers (3, 4) deal with the towards the lignin content and structure. Two recent attention in these hardwoods has been directed mainly European aspens respectively) have not been extensive. All des Michx. and Populus tremula L. (the North American and investigations into the extractives from Populus tremula - Populus has lasted nearly one hundred and thirty years,

In spite of the fact that interest in the genus

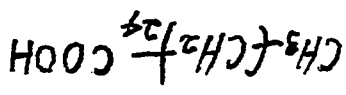
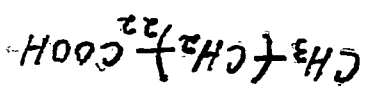
The first detailed study into the components of poplar extractives was made by Hossfeld and Hunter, working with aspen bark (10). These workers were successful in isolating linoleic (C₁₈) and lignoceric (C₂₄) acids, β-sitosterol (VIII), ceryl alcohol (C₂₄) (IX), glycerol, a β-hydroxy-sterol, and a hydrocarbon.

(9): again, no individual compounds were isolated or identified. Work on the wood bark was carried out by Chang and Mitchell regard to the nature of the compounds present. Similar various fractions. Nothing, however, was indicated with vents, the iodine numbers, and related properties of the the percentage of extractives obtained with various sol- ted systematically by Browning (8). This work dealt with North American aspen seems to have been first extrac-

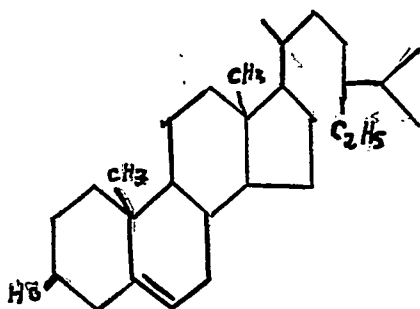
3. Populus tremuloides Michx.

Lignoceric Acid
(Tetracosanoic Acid)
(VII)

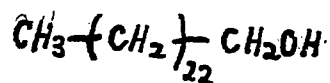
Cerotic Acid
(Hexacosanoic Acid)
(VI)



eral extract of aspen wood (7). (C₂₆) (VI) and Lignoceric (C₂₄) (VII) acids, from the eth- lated sitosterol glucoside, C₃₅H₆₀O₆, as well as cerotic components of the extractives. Perilla claimed to have iso-



β -sitosterol
(VIII)

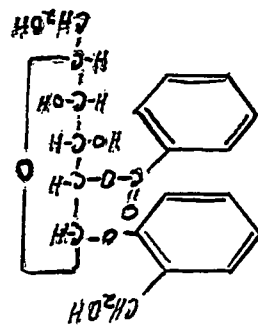


Ceryl alcohol
(IX)

In a study of North American pulpwoods, Buchanan, Sinnott, and Jappe (11) found among the constituents of aspen wood all the even-numbered normal fatty acids from palmitic (C_{16}) to lignoceric (C_{24}) acids and identified them by means of paper chromatography. In addition to these acids, the above authors determined the presence of linoleic and traces of linolenic acids among the acid components. Palmitic acid was found to be the most abundant fatty acid in the extracts.

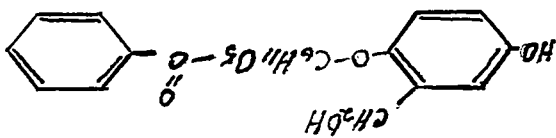
Recently, Pearl and Darling (12), working with aspen bark, isolated a new glycoside, tremuloidin, shown to be 2-benzoylsalicin (X) and salireposide (XI) (12a).

Tremuloidin
(X)



(XI)

Salitreposide (proposed)



Although well over fifty-six per cent of Saskatchewan's land is covered by forest (not considering the aspen grove area) (13), forestry ranks fourth in the list of the province's industries, bringing in an annual gross of only about ten million dollars. This figure is small indeed when compared with the seven hundred and forty-three million dollars earned in farming on a smaller land area in 1953. The above, of course, does not consider the value of the forests in regard to being an encouraging habitat for fur-bearing animals and game birds and animals as well as being a very serviceable environment in resort areas.

The Department of Natural Resources of the Province of Saskatchewan has predicted that within the next two decades forest products will have a gross annual product of over one hundred million dollars (14). This, however, does not necessarily mean that any major share of this figure will be derived from poplar. Even though the aspen is the province's most abundant tree (15, 16, 18), peak years did not find poplars contributing much more than twenty per cent of the province's lumber output (18). At present there is only one sawmill in Saskatchewan producing poplar

1. The Resource and Potential

II. POPLAR AS A SASKATCHEWAN NATURAL RESOURCE

This plant has a maximum annual capacity of only two million board feet. The only other outlets for poplar in this province are two plywood factories and a fibreboard factory which utilize an annual maximum of 6,000 and 5,000 cords, respectively (18).

The forestry industry in Saskatchewan has the potential to surpass the one-hundred-million-dollar gross production for it. However, great expansion will be required in poplar utilization. One obvious method, aside from the expansion of existing operations, is the establishment of local pulp and paper industries for the utilization of poplar, a method of utilization found highly successful in the United States (17). Coupled with these possibilities is the increasing inaccessibility of pure poplar stands. There is also the difficulty of getting logs large enough in diameter, and sufficiently free of decay, for economical production of forestry products. This latter point is a very major consideration, indeed, in tapping Saskatchewan's forest potential.

III. OBJECT OF THE RESEARCH

The aim of the research was to initiate the systematic investigation of the extraneous materials, the unbound extractives, of the healthy heartwood of the aspen tree, Populus tremuloides.

The first part of the investigation was undertaken to determine a method of extracting the ground heartwood which would (1) give consistent percentages of extractives, (2) yield extractives of relatively constant composition, and (3) afford preliminary fractionation of the groups of extractives rather than the impregnable mass of materials and compounds which is often encountered in solvent extraction of plant products.

The second section of the research was to deal with the isolation, characterization, and identification of individual compounds, with the possible end in mind that they might be of use in industry as a precursor or in their natural form.

Before the work which will be outlined in Extraction Schemes I and II was undertaken, extraction experiments employing small lots of wood (approximately 50 g.) were carried out in an attempt to develop a systematic approach to this problem so that extractives of constant composition and in constant yields would be obtained. Extraction of the wood with petroleum ether (b.p. 40-60°), ether, and steam distillation of the wood in various orders did not appear to give different products as indicated by the infra-red spectra of the individual fractions. At best, only mixtures of variable composition were obtained; the yields were not reproducible either. For example, the yields of the steam volatile matter obtained from unextracted wood may be cited. Yields of this material varied from a high of 1.38% to a low of 0.03%. The reasons for such a wide variation were not investigated. In the above case, however, the difference in yields could have been due to differences in extraneous matter composition of individual lots of wood—a factor which should have been minimized by the mixing of wood meal from a large number of source wood bolts and therefore seems highly unlikely—or

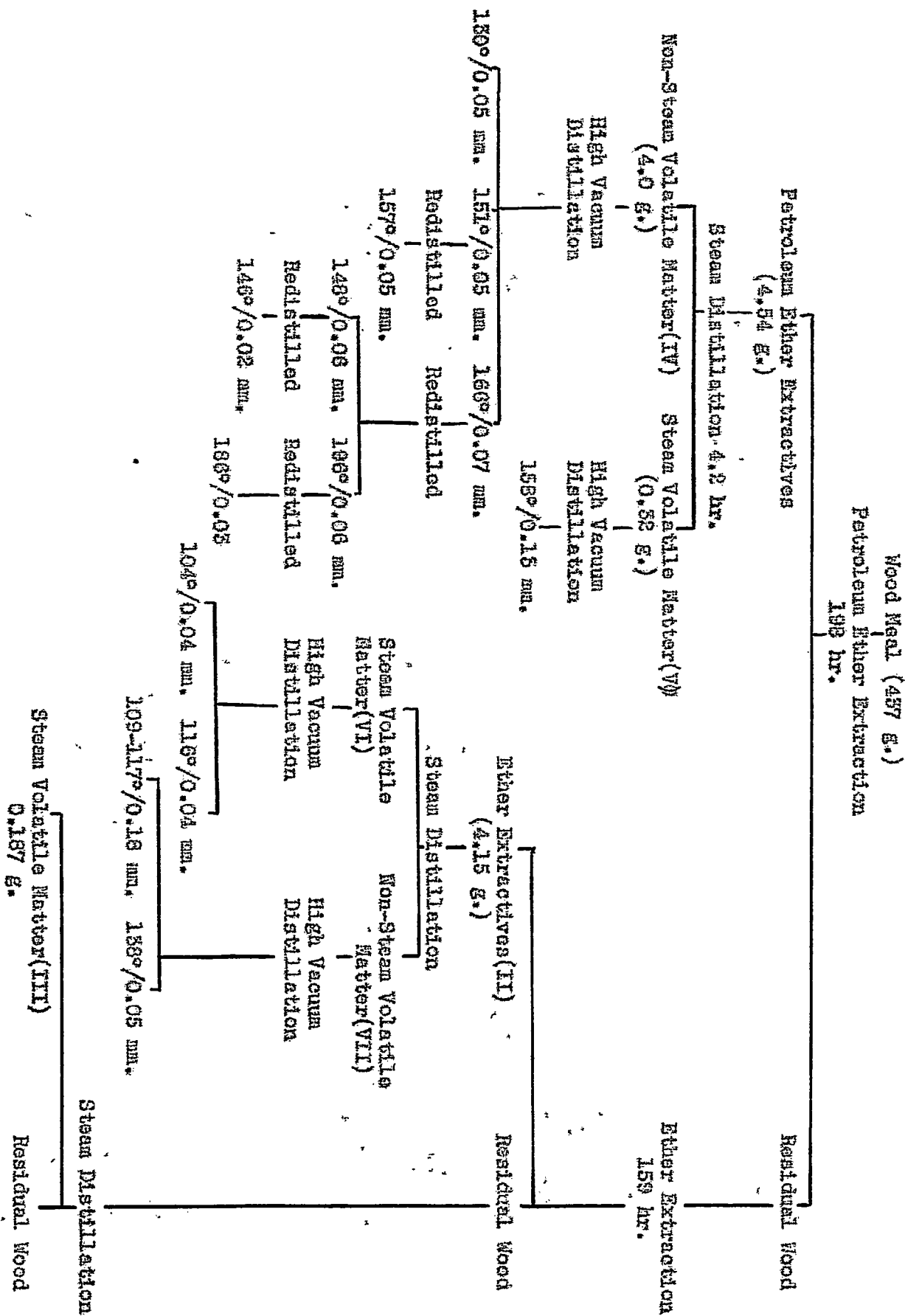
1. Preliminary Extraction Schemes

IV. DISCUSSION

to changes in pH of the steam (directly from the steam line) used in the distillation, resulting in the hydrolysis of steam volatile compounds to non-steam volatile forms. This does not entirely explain the differences since Extraction Scheme I was found to give a total of less than 0.2% of steam volatile matter employing steam generated from distilled water. Thus even when the factor of pH was eliminated a batch of wood gave a yield of steam volatile matter only one-seventh as great as its counterpart in Extraction Scheme II* and III) were undertaken in the expectation that the solvents would give rise to at least partial fractionation of the extraneous matter in the heartwood and that the steam distillation of the extractives thus obtained would further separate the components into fractions containing relatively few components by removing free fatty acids below C₈ (46) and other steam volatile matter that might be present. It was hoped that the final step, which involved fractional distillation by high vacuum distillation, would complete the task of separation and purification. When the extractions and distillations of the first scheme were carried out (see flow sheet I), the very similar nature of the crude extractions and the final distillates, as evidenced from a comparison of the infra-red spectra of the various products

Extraction Schemes I and II (see flow sheets I, II, and III) were undertaken in the expectation that the solvents would give rise to at least partial fractionation of the extraneous matter in the heartwood and that the steam distillation of the extractives thus obtained would further separate the components into fractions containing relatively few components by removing free fatty acids below C₈ (46) and other steam volatile matter that might be present. It was hoped that the final step, which involved fractional distillation by high vacuum distillation, would complete the task of separation and purification. When the extractions and distillations of the first scheme were carried out (see flow sheet I), the very similar nature of the crude extractions and the final distillates, as evidenced from a comparison of the infra-red spectra of the various products

Extraction Scheme I



EXTRACTION SCHEME II

Wood Meal (437 g.)

Steam Distillation

Steam Volatile Matter (A) (6.07 g.)

Hydrolysis

SEE FLOW SHEET III

Paper Chromatography of Acids (AA)

Petroleum Ether Extraction

Residual Wood

Residual Wood

Petroleum Ether Extractives (B) (5.82 g.)

Steam Distillation

Non-Steam Volatile Matter (D) (0.63 g.)

Matter (E) (4.55 g.)

High Vacuum Distillation

98°/0.1 mm. 175°/0.9 mm.

High Vacuum Distillation

150°/0.09 mm. 188°/0.1 mm. 206°/0.1 mm.

Ether Extractives (c) (1.06 g.)

Steam Distillation

Non-Steam Volatile Matter (H) (0.909 g.)

Steam Volatile Matter (F) (0.09 g.)

Residual Wood

Steam Distillation

Residual Wood

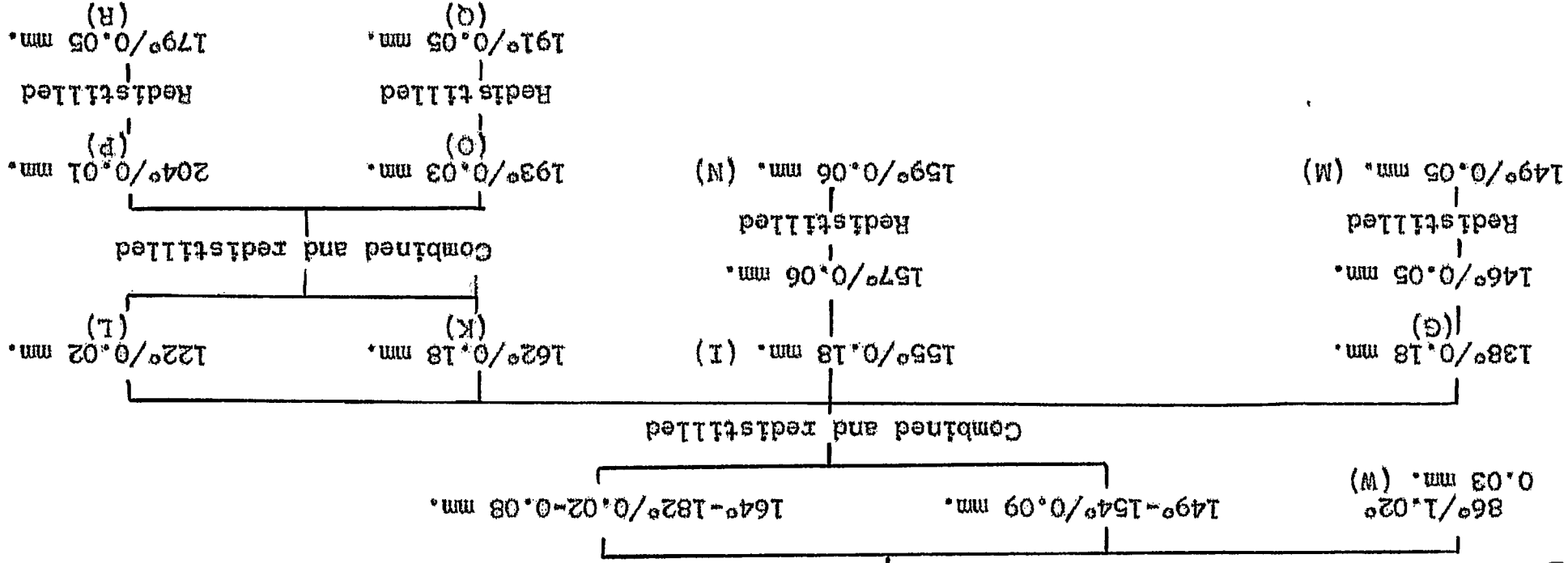
Steam Volatile Matter (4.2 mg.)

FLOW SHEET II

EXTRACTION SCHEME III--STEAM VOLATILE MATTER

Steam Volatile Matter of Wood (6.00 g.) (A)

High Vacuum Distillation



FLOW SHEET III

and the starting material, led to the conclusion that the scheme was not leading to any satisfactory separations. Analysis of some of the individual fractions also led to variable and non-reproducible results. Consequently, the scheme was set aside.

In carrying out Extraction Scheme II, results seemed considerably more promising. In the final stages, high vacuum distillation gave sharp fractions, which were satisfactorily redistilled and purified, while the infra-red spectral tracings also indicated that fractionation was being achieved. Microanalyses of a number of supposedly pure samples, however, were unsatisfactory and found to be unreasonable when it was noted that some of the higher boiling cuts gave analyses expected for compounds of lower molecular weights than would have been possible (on the basis of the boiling points). The limitation of Extraction Scheme II, insofar as the isolation of pure individual compounds was concerned, was further demonstrated when portions of three different supposedly analytically pure samples (G, I, and P) were introduced into a 4' silicone-on-firebrick column in a gas chromatography unit at 190° (helium inlet pressure 25 p.s.i.) and were found to be composed of mixtures of 5, 7, and 9 compounds, respectively. It was thus felt that Extraction Scheme II was not

sufficient to separate individual components out of the heartwood extractives. The study of further fractions was not undertaken.

2. Extraction, Separation, and Fractionation of the Free Fatty Acids (See Flow Sheet IV)

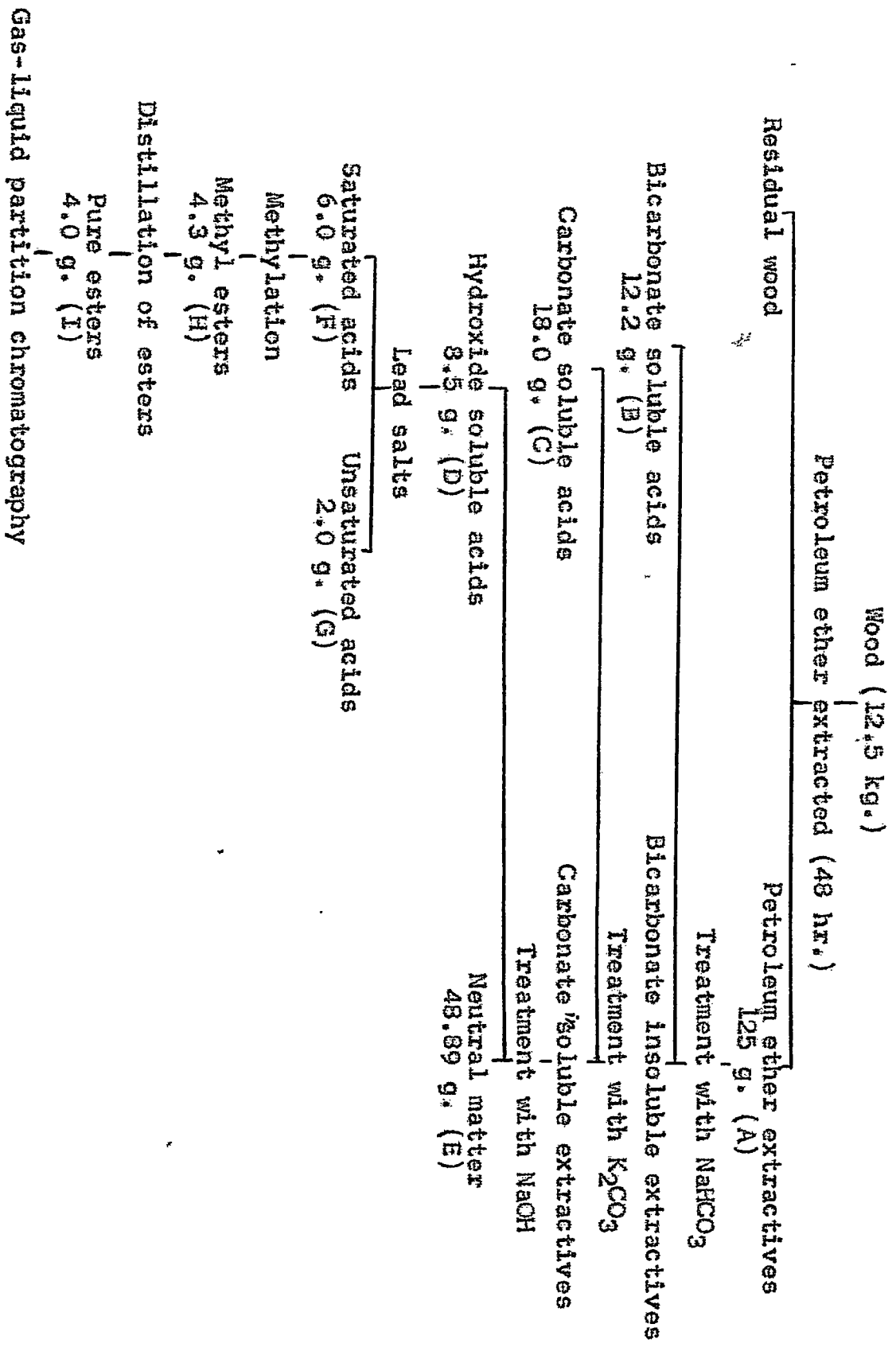
(a) Extraction

It was concluded after the extraction schemes had been attempted that little or no separation of the extractives could be resolved by changing the solvent used for the extractions. It was therefore decided to choose petroleum ether (b.p. 40-60°) as solvent for the extractions since the extraneous matter extracted with this solvent seemed to be most free of dark-coloured resinous matter and contained a minimum of material which would not distil under high vacuum. To minimize the effect of variations in the wood composition and, to a lesser extent, variations in extraction conditions (packing of the wood in the Soxhlet chamber, small variations in extraction time, and so on), a number of lots of wood were Soxhlet extracted without changing the petroleum ether in the still. In this manner, 1% petroleum ether extractable material was consistently obtained. In every case, the extractives were light orange in colour and had the consistency of a heavy oil.

(b) Separation

It was noted in handling the petroleum ether

EXTRACTION, SEPARATION, AND FRACTIONATION OF FREE FATTY ACIDS



extractives that washing of the extractives with solutions of various strength alkalis gave arbitrary separation of the acids present into groups of soaps and a neutral fraction. Exhaustively washing the petroleum ether extractives in turn with 5% aqueous solutions of sodium bicarbonate, potassium carbonate, and sodium hydroxide gave 16.5% bicarbonate soluble, 19.65% carbonate soluble, and 9.27% hydroxide soluble acids based on the total weight of extractives. The neutral matter, namely, the esters, accounted for 53.37% of the extractives.

The sodium hydroxide soluble acids were chosen for detailed study, even though they represented the smallest fraction, since it was felt that the resin acids and a good deal of the low member and unsaturated fatty acids would have been removed by the washing with the weaker alkalis, resulting in the sodium hydroxide soluble acid fraction being relatively uncomplicated.

The sodium hydroxide soluble acids were further fractionated into a saturated and an unsaturated fraction by means of the lead salt technique introduced by Twitchell (19) in 1921. To make the separations obtained by this procedure as sharp as possible, the insoluble lead salts were twice recrystallized from ethanol, giving 75% of the total sodium hydroxide soluble acids as saturated acid salts and 25% as unsaturated acids.

(c) Fractionation

Although a good deal of the fractionation of the fatty acids had already been carried out, and because of the relatively small amount of fatty acid mixture on hand, and especially in light of the observations on the unreliability of high vacuum distillation in the fractionation of the products obtained in Extraction Schemes I and II, it was decided to attempt the separation and identification of the saturated sodium hydroxide soluble fatty acids, in the form of their methyl esters, by gas-liquid partition chromatography. A standard Beckman G.C.-2 gas chromatography unit was employed. New columns were prepared for this work, employing standard techniques for both the silicone-on-C₂₂ firebrick (20, 21) and polyester-on-Celite 545 (22) columns. The gas chromatography apparatus is diagrammatically described below (see Fig. 1). A modified injector system, heated by means of a heating tape to 230°, was used (see Fig. 2) at the head of the column and this gave much better results than with the standard commercial injector.

The approach to the analysis of the composition of the methyl esters of the sodium hydroxide soluble saturated fatty acids was standard. The silicone column was used first to resolve the esters according to their carbon chain length (59, 60) and thus determine how many members of the