

Studies on Wood Phenolics (II)^{*,**}

Extractives from Heartwood of *Metasequoia glyptostroboides*

HU et CHENG (Part 1)

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木材フェノール性化合物に関する研究 (第2報)^{*,**}メタセコイアの
心材抽出成分について (その1)

Introduction

The tree, *Metasequoia glyptostroboides* HU et CHENG, has been introduced as a kind of fossil tree by Dr. S. Miki (Osaka, Japan; 1941), and Mr. Wang Tsang has discovered it growing in Province of Szechwan (Mainland of China; 1945)¹⁾. It is commonly known that *Metasequoia* is a tree of high growth rate.

No survey for the minor constituents of heartwood like resins or phenolics has been found except several papers^{2)~6)} concerning leaf-extracts. On the other hand, preliminary test for preparing of pulp from this tree was done by T. KOSHJIMA, K. KITAO and I. TACHI⁷⁾, but they did not check about the extractives.

Chemical analysis for minor constituents was applied to the heartwood to know the chemo-taxonomical situation among the species of trees and how this tree will be useful for wood industry in future. Many results were mainly obtained by comparison with data from both GLC and TLC. Through the GLC experiments, trimethylsilyl (TMS) derivatives were favorable for polyhydroxy-compound like phenolic acids and cyclitols.

Experimental and Results

Wood The thirteen years old tree planted in the yard of Experimental Forest Station of Kyoto University (Kitashirakawa, Kyoto) was cut down in the Winter, and the heartwood portion was used as the sample after milling.

Analytical instruments Both gas-chromatographs, Shimadzu GC-1 B and Yanagimoto GCG-3DH were operated under flowing of helium gas, and several different

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Table 1. Varieties of Columns Fixed on the Instruments.

Fraction	Instrument, unit of column	Shimadzu G1-1B U-types stainless 6mm ϕ , 75cm	Yanagimoto GCG-3DH γ -type stainless 6mm ϕ , 1m
steam distillate		P. E. G. 4000 on Chr*.	
hydroxy compounds (phenolics, cyclitol)		5% DC 430 on Celite** 30% DC 430 on Celite 1% XE 60 on Chr.	5% DC 430 on Chr. 30% DC 430 on Chr. 1% EX 60 on Chr.
fatty acids		30% Butanediol succinate (Craig's ester) on Chr.	

* Chromosorb W. (60~80 mesh) was used.

** Celite 545 (30~50 mesh) was used.

columns shown in Table 1 were fitted. Thermal conductivity detector was attached to the each instrument. Among the columns, series of DC 430 were prepared in our laboratory as reported previously⁸⁾, and others were commercial. All TLC equipments, similar to Desage Type, were purchased from Yazawa Co. Thin-layers of Silica Gel G (Merck) were adjusted to 250 μ . Ferric chloride (as 1% soln.),

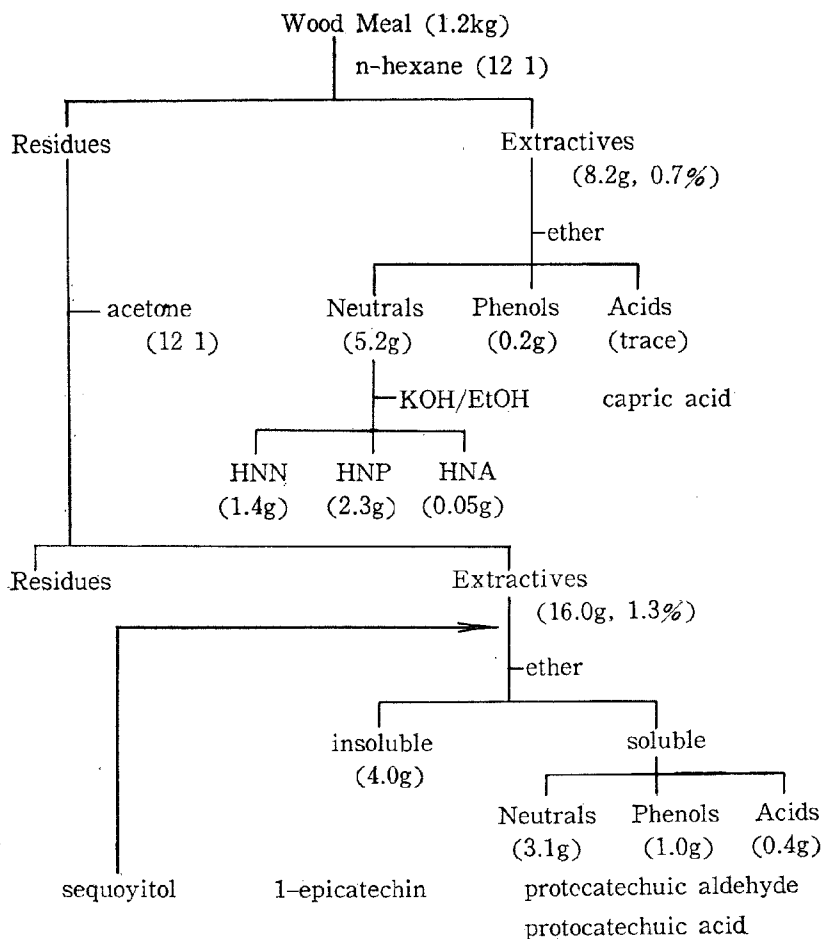


Fig. 1. Fractionation from Metasequoia (heartwood).

diazotized benzidine, and acidic solution of potassium permanganate used by E. von RUDLOFF and A. SATO⁹⁾ were used for spraying reagents of TLC. MN-cellulose G was also useful for TLC of sequoyitol and this plate was developed with acetone-water (4 : 1 v/v) and sprayed ammoniacal silver nitrate solution according to L. ANDERSON et al¹⁰⁾.

Fractionation Air dried wood meal (40 to 80 mesh ; 1.2kg) was packed in a wide-mouthed bottle (10 l). Total 12 l of n-hexane was used for extraction by three times, and the same volume of acetone was followed as same procedure described above. Extraction were done at a room temperature. Each solution was concentrated at reduced pressure to give syrup by using a rotary evaporator. The syrup was extracted with ether and the ether soluble portion were divided into following three groups ; the acid soluble in 5% NaHCO₃, the phenols soluble in 2N-NaOH, and the neutral substances. The yields are shown in Figure 1.

Steam distillate A hundred gram of wood meal packed in 500 ml round flask

Table 2. Steam Distillate from Metasequoia (heartwood).

compound (r. r. t.)	sample (r. r. t.)
α -pinene 0.18	<u>0.18</u> (α -pinene)
camphene 0.22	<u>0.26</u>
limonene 0.32	0.33
cineol 0.36	0.46
	0.54
	<u>0.66</u>
linalol 1.00*	
terpineol 2.00	<u>2.00</u> (terpineol)
	2.22
benzyl acetate 2.38	
	<u>2.50</u>
benzyl alcohol 3.90	
	<u>5.60</u>

PEG 4000, 1.5m, 160°C, 120 ml. He

* t_R3.8 minutes

(yield, 0.14%). The compound was melted at 243~245°C, and the value of relative retention time (r. r. t.) as the TMS derivative against TMS-vanillic acid (retention time 3.4 min.) was 2:82 according to the method of A. SATO, K. KITAO and M. SENDA⁹⁾.

In this case, 5% DC 430 column (0.75m) was chosen and operated at 160°C.

was distilled with steam for two hours. The distillate was collected in acetone which was cooled with ice. Acetone solution diluted with distilled water was extracted with ether and the concentrate of ether extract was injected on the instrument. The instrument equipped with a column of PEG 4000 was operated at 160°C. As shown in Tale 2, the results are compared with several authentic compounds. α -Pinene and a small peak of terpineol coincide with authentic, but other four big and two small peaks are unidentified.

Isolation of sequoyitol Fine crystals of leaflet were first observed when the acetone extract obtained from 10g of wood meal by using a Soxhlet extractor was kept in cold. Fourteen mg of crystal was collected from the above

Data of TLC from cellulose plates were also satisfactory, because the value of R_f was 0.26 in the mixed solvent of n-butanol, acetic acid, and water (4 : 1 : 5 v/v). These data coincided with the compound which was synthesized at Nakajima's laboratory. Analysis gave the following results :

Calcd. for C₇H₁₄O₆ : C, 43.29%, H, 7.27%

Found : C, 43.10%, H, 7.30%

Another 100 mg of the compound was mixed with 150 mg of anhydrous sodium acetate and dissolved into 120 ml of acetic anhydride and heated for two hours with a reflux condenser. 139 mg of the acetate was obtained and melted at 196°~198°C after recrystallizing from ethanol. (Lit.¹¹ for sequoyitol pentaacetate 198°C).

Anal. Calcd. for C₁₇H₂₄O₁₁ : C, 50.49%, H, 5.98%

Found : C, 50.50%, H, 6.08%

Infrared spectra for sequoyitol and its acetate were satisfactory as compared in Figure 2.

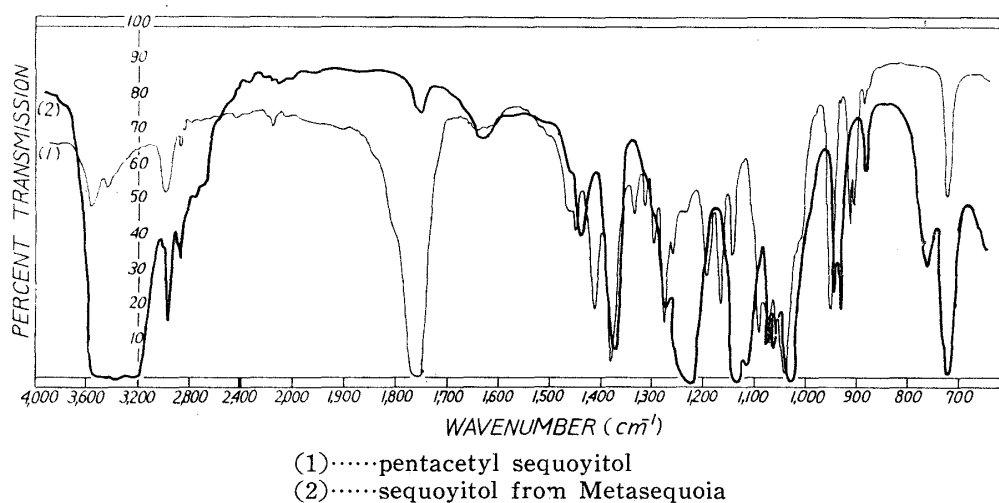


Fig. 2. I. R. spectra of sequoyitol and its acetate from Metasequoia.

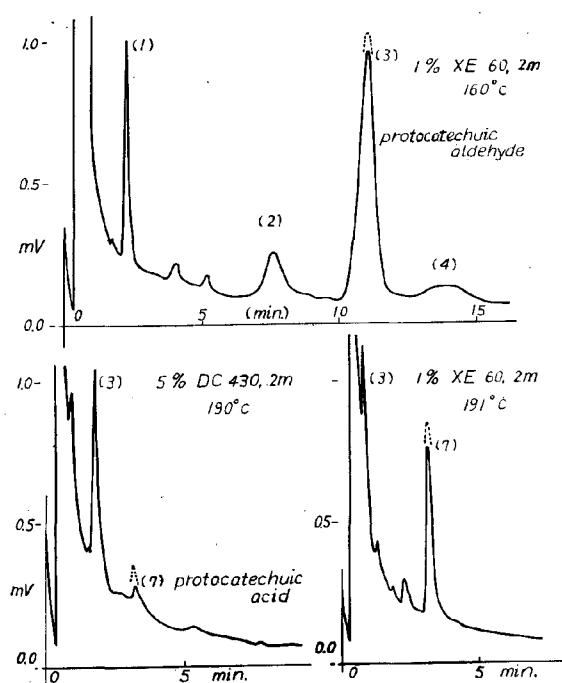
Hexane soluble fraction Hexane soluble portion was divided into three groups as described above. The acid portion was directly methylated with ether solution of diazomethane, and injected to GLC in which the column of butanediol succinate was fixed. The neutral portion being major part of hexane soluble substance was saponified in alcoholic alkaline solution and resultant acids were taken in ether, treated with diazomethane, and the methylated sample was used for GLC under the same condition. From the results shown in Table 3, capric acid is identified in the acid portion, and the relative retention times of capric, lauric, myristic and palmitic acids coincide with authentics. Among the compounds, palmitic acid is most abundant and an unidentified compound supposed to be a C-13 fatty acid is also recorded.

Table 3. G. L. C. of Fatty Acids from Metasequoia (heartwood).

compound	r. r. t.	sample (methylated) r. r. t.	
		free acid	combined acids
methyl caprylate (C 8)	0.30	—	—
// caprate (C 10)	0.55	0.55	0.55
// laurate (C 12)	1.00*	—	1.00
		—	1.55
// myristate (C 14)	2.05	—	2.05
// palmitate (C 16)	3.60	—	3.60
// stearate (C 18)	6.25	—	—

Craig's Ester 1.5m, 190°C, 120 ml He

* t_R2.0 minutes



Dotted line shows the peak due to authentic compound.

Fig. 3. Chromatograms of Ether Soluble Fraction (acids).

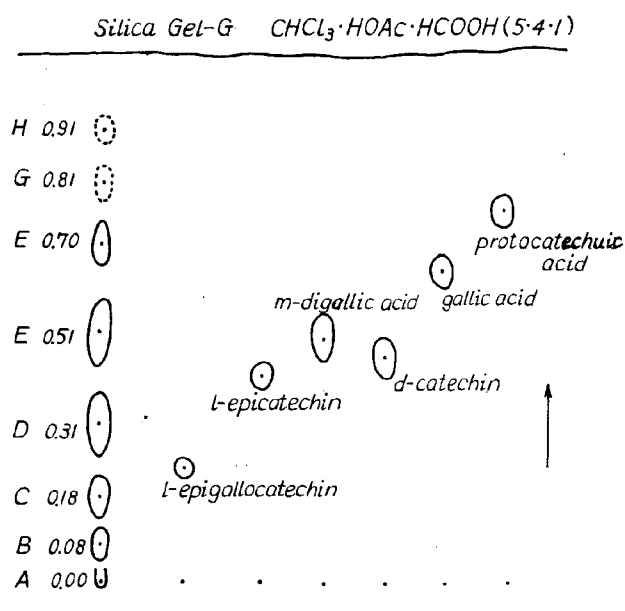


Fig. 4. TLC of Acetone Soluble Fraction.

Ether soluble fraction Ether soluble portion of acetone extract was also divided into acids, phenols and neutrals as Figure 1. Eight different spots appeared on the TLC-plate (Silica Gel G) when a mixture of benzene, dioxane and acetic acid (90 : 25 : 4 v/v) was used as developer, and sprayed ferric chloride solution. Among the spots the blue spot having 0.33, value of R_f (in Figure 3), was strongly suggestive of protocatechuic aldehyde. Another data from GLC about this fraction clearly confirmed the presence of protocatechuic aldehyde and protocatechuic acid derived

to TMS derivatives. (in Figure 4).

Acetone soluble fraction Ether insoluble portion (pale brown) had eight spots on the Silica gel plate developed with a mixture of chloroform, acetic acid and formic acid (5 : 4 : 1 v/v) and coloured with the benzidine reagent. The spot named E (Rf-value, 0.51 as shown in Figure 4) was supposed to be one of *m*-digallic acid, *d*-catechin or 1-epicatechin. The data from GLC concerning this fraction gave preciser answer as shown in Figure 5. 1-Epicatechin and *d*-catechin gave clear sepa-

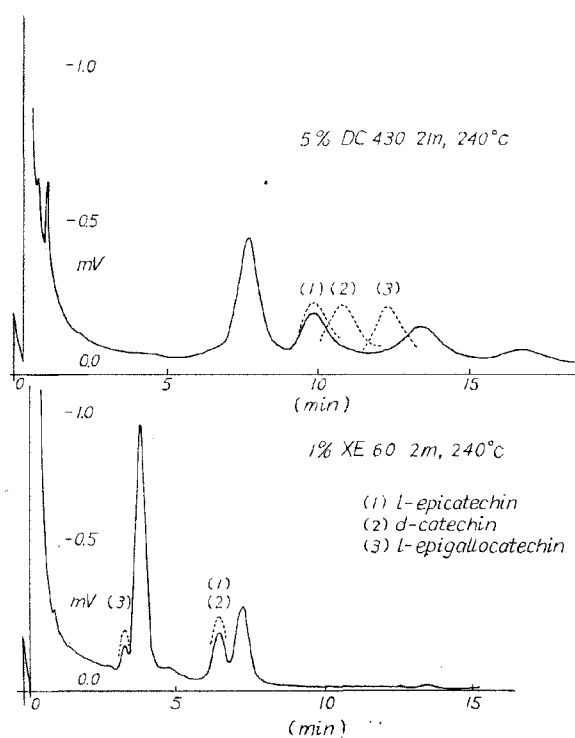


Fig. 5. Chromatograms of Acetone Soluble Fraction.

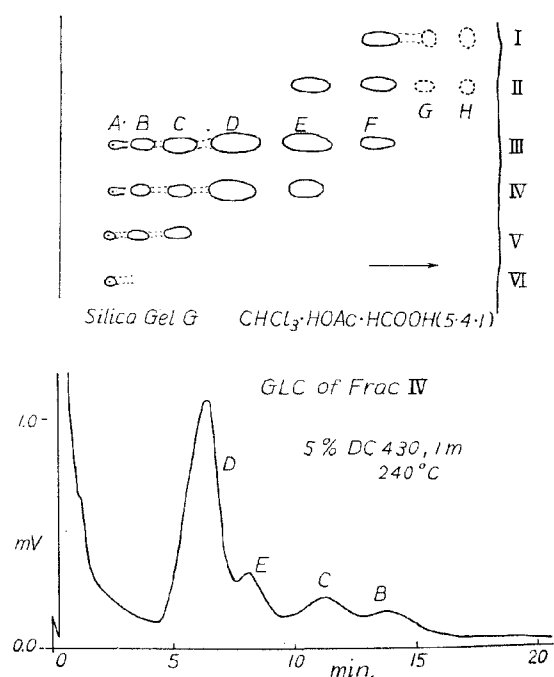


Fig. 6. Comparison between both Chromatograms. (Acetone Soluble Fraction)

rate peaks each other by using 5% DC 430 or 1% XE 60 column, but *m*-digallic acid gave no peak in the form of TMS derivative. The Fraction IV collected through the cellulose column as described below showed the peaks of Figure 6 in which TMS derivatives were injected in the instrument. Among these peaks, one named D had the largest area on the TLC, and the peak matched with the highest peak of GLC. The compound named E was confirmed as 1-epicatechin from the data of Rf-value and r. r. t.

Twenty grams of cellulose powder were suspended in 50 ml of distilled water, and poured into a glass column (d., 2 cm, l., 50 cm). Two grams of ether insoluble fraction dissolved in the mixture of chloroform and ethyl acetate (1 : 3 v/v) was placed on the column, and eluted with the solvent system shown in Table 4. Every 40 ml of eluate was collected and checked by TLC after concentration.

Table 4. Fractionation of Acetone Soluble Fraction.

Fraction	Composition of eluate (each 40 ml)	Yield (g)
I	chloroform : ethyl acetate (1 : 3)	trace
II	"	0.32
III	ethyl acetate	0.70
IV	ethyl acetate : acetone (1 : 1)	0.30
V	acetone	0.25
VI	methanol	0.15
	total	1.72

Methanol extract Methanol extract was obtained in a yield of 3.4% from 2 kg of wood meal by using the same method as described above. In the extract, a compound (6.88g) which was insoluble in hot water or chloroform but soluble in acetone or methanol was obtained. This pink amorphous compound was softened at 199°C. Its acetate prepared with acetic anhydride and pyridine was colourless and melted at 121°~122°C.

Analysis of the former gave the following results :

Found : C, 62.81%, H, 6.38%

Calcd. for $(C_{19}H_{23}O_7)_n$: C, 62.9%, H, 6.32%

Infrared spectrum of this compound is shown in Figure 7. Further investigation

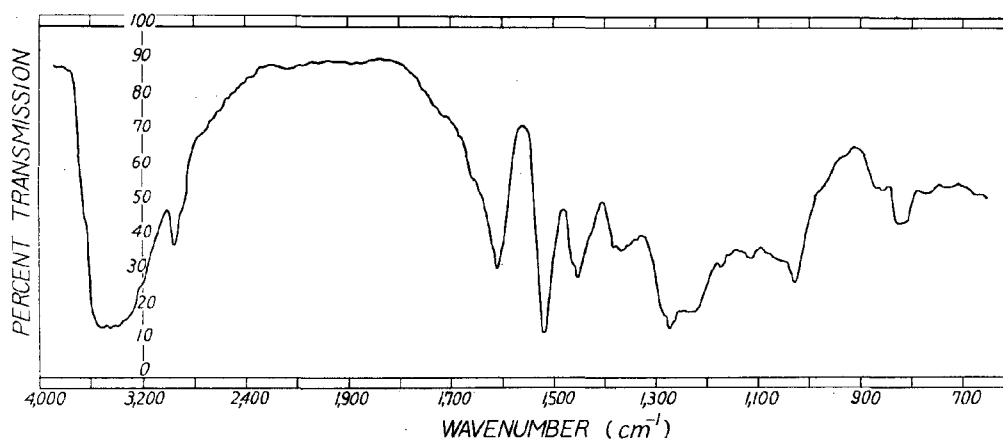


Fig. 7. I. R. spectrum of a compound from methanol extract.

is in progress.

Discussion

Sequoyitol is first isolated from the heartwood of *Metasequoia glyptostroboides*, although the presence of this compound in leaves was reported before⁴⁾. Meanwhile, d-pinitol and sequoyitol among cyclitols are well known constituents in leaves or trunks of conifer trees. In many cases, both compounds were found together in

trees like *Sequoia sempervirens* and several kinds of pines¹²⁾. In this time, only sequoyitol was isolated from the tree and no spot due to d-pinitol was detected. These facts are very interesting in point of chemo-taxonomical distribution of cyclitols among the trees.

Great advances of TLC-techniques have been recently performed, but further advance is expected by additional uses of GLC. For instance, the identification of l-epicatechin, d-catechin and *m*-digallic acid was difficult on the TLC-chromatogram from each other, but the last one showed no peak on the GLC-chart by our TMS method, and l-epicatechin and d-catechin had different retention times, namely 9.0 minutes for the former and 9.9 for the latter on the described condition. Thus, usefulness for trimethylsilyl derivatives in the field of polyphenolic compounds is reconfirmed through present experiments.

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Summary

The chemical constituents of heartwood of *Metasequoia glyptostroboides* were mainly analyzed by gas-liquid chromatographic (GLC) and thin-layer chromatographic (TLC) techniques. Capric, lauric, myristic, and palmitic acids were found in the hexane soluble portion with an unidentified acid as esters. Capric acid was found also as a free form. Sequoyitol was isolated from the acetone soluble fraction in the yield of 0.14% based on air dried wood meal. Protocatechuic aldehyde and protocatechuic acid were identified by TLC and GLC in the ether soluble fraction. l-Epicatechin was also identified among total eight constituents in the acetone soluble fraction. The phenolic compounds and the cyclitol above mentioned were successfully applied to GLC as trimethylsilyl derivatives by using DC 430, a silicone rubber, column. Total ten peaks were recorded on the GLC-chart from the steam volatile, and two of them were identified as α -pinene and terpeneol. In the methanol extract, a pink amorphous compound ($C_{19}H_{23}O_7$)_n, m. p. 199°C, was isolated in the yield of 0.3%.

摘 要

メタセコイア心材から、はじめてセコイトール (0.14%) が単離され、他に α -ピネン、テ

ルピネオール, カプリン酸, プロトカテキン酸とそのアルデヒド, と 1-エピカテキンの存在を, またカプリン酸, ラウリン酸, ミリスチン酸, パルミチン酸の各エステルが存在を主にガスクロマト法と薄層クロマト法を併用して確認した。また未同定化合物 ($C_{19}H_{23}O_7$)_n, m. p. 199°C, を0.3%の収量で得た。ポリフェノール化合物へのトリメチルシリル誘導体の有用性が再確認された。

Literature

- 1) MIKI, S., "On Metasequoia, Fossil and Living", p. 53, Nippon Kobutu Syuminokai, Kyoto, Japan (1953).
- 2) HIDA, M., Bot. Mag. Tokyo, 71, 425 (1958).
- 3) HIDA, M., and K. IDA, *ibid.*, 74, 369 (1961).
- 4) KARIYONE, T., K. TAKAHASHI, K. ISOI and M. YOSHIKURA, J. Phar. Soc. Japan, 78, 801 (1958).
- 5) TAKAHASHI, M., T. ITO and A. MIZUTANI, *ibid.*, 80, 1557 (1960).
- 6) ISOI, K., *ibid.*, 78, 814 (1958).
- 7) KOSHIJIMA, T., K. KITAO and I. TACHI, Wood Research, No. 13, 150 (1954).
- 8) SATO, A., K. KITAO and M. SENDA, *ibid.*, No. 34, 237 (1965).
- 9) von RUDLOFF, E. and A. SATO, Can. J. Chem., 41, 2165 (1963).
- 10) ANDERSON, L., E. S. DELUCA, A. BIEDER and G. G. POST, J. Am. Chem. Soc., 79, 1171 (1957).
- 11) SHERRARD, E. C. and E. F. KURTH, *ibid.*, 51, 3139 (1929).
- 12) PLOUVIER, V., "Chemical Plant Taxonomy" Ed., T. Swain, p. 327, Academic Press, London (1963).