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A Study of the Chemical Constituents of Gymnosperm pollens

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The chemical compositions of pollen have been studied in relation to human allergic responses (Knox et al 1970; Lewis et al., 1967), human diet, bee nutrition(Watabe et al., 1961), self-incompatibility (Howlett et al., 1975) and taxonomy(Lawrence, G.H., 1951) etc. Endogenous chemicals in pollen have been correlated with patterns of inheritance and growth requirements for pollen(Nielsen et al., 1955; Shaw et al., 1968). Growing pollen *in vitro* also permits the study of chemicals required for growth process such as nuclear division and cell wall extension.

The particular interest in pollen is growing due to the fact that it is now possible to produce the adult haploid plants from the culture of isolated pollen grains in chemically defined media, permitting geneticians to select new physiological mutant-plants with a given character similar to handling microorganisms by microbiologists (Nitsch, 1974).

In gymnosperms, microspores are formed in the microstrobili which generally develop in the axile of scale leaves near the tips of branches. Each macrostrobilus usually bears many microsporophylls in a spiral arrangement around the central axis, each with two or more microsporangia on the lower side. Microspore mother cells undergo meiosis giving rise to the spore tetrad, each yielding 4 microspores, ultimately the pollen grains. Each pollen grain is sealed in a double layered wall. The outer pollen wall in many species forms two conspicuous wings or sacs which contain air. Such structures reduce the free fall velocity of these wind-dispersed, anemophilous pollen. Gymnosperm pollens show the complete gametophytic development pattern; the nuclei divide several times and resulting mature grains, in Pinus for example, contain the following cells; two non-functional prothallial cells, a central vegetative or tube cell and the generative cell, the latter two originating from the antheridial initial. With formation of these cells, gymnosperm pollen grains are shed from the microsprangia.

The generative and the vegetative cells have been shown to synthesize RNA and proteins during germination and tube growth and have been studied on the specific roles they perform during that period(LaFountain et al., 1972; Mascarenhas et al., 1969). Though many of their specific roles have not been known, the destiny of the generative cells has been known to be gamete formation and the main role of the vegetative cell seems to be related to tube growth (Pipkin et al., 1973). The male gametophytic or sperm cells which are formed by generative cell division in pollen tube are ready to fulfil their function in fertilization. The sperm cells participate in double fertilization in angio-

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sperms, one forming the 2n zygote, the other usually giving rise to the 3n endosperm. In gymnosperms one sperm cell generally disintegrates; the other produces a 2n zygote.

We have previously repoted in a preliminary study the distribution of DNA, RNA, lipid phosphorus, triglycerides, residual nitrogen, and acid-soluble phosphorus in pollens from 13 species of gymnosperm and angiosperm plants (Lee and Chung, 1976).

In a serial work, we present this time a study on the chemical compositions of pollens in special reference to their carbohydrates and lipid patterns, in exclusive gymnosperm species and one angiosperm species as comparison.

MATERIALS AND METHODS

Collection of pollen grain and sample preparation

Bunch of full-blown flowers concerned were placed on a clean sheet of paper and pollens detached from anthers spontaneously or by mechanical shaking were taken and sieved to obtain homogeneous grain size. Special precaution was taken to the collection of pollen grains free of dust and microorganisms.

Pollen grains of individual species were disrupted using French press (Aminco). Suspension (10%, w/v) of pollen grains in distlled water was introduced into French press cell and pressure was brought to 20,000 psi. Liquid containing disrupted pollen grians was allowed to escape dropwise through the exit valve and was collected, confirming rupture of cell walls by microscopy and lyophilized immediately. The lyophilized powder of pollen grains was preserved in a short time in a desiccator filled with nitrogen gas until chemical analyses.

Estimation of nucleic acids

500 mg of the lyophilized sample were homogenized in an appropriate amount of 5% perchloric acid at 0°C, allowing to extract the acid-soluble material and centrifuged. This process was repeated twice. The supernatant was then discarded to remove the acid-soluble fraction and the residue was delipidated withm ethanol and subsequently with alcohol-ether (3:1).

The total nucleic acid content was determined by measuring UV absorbance of the extract at 260 nm and 290 nm(Cherry, 1973). DNA content in the extract was determined spectrophotometrically by use of the diphenylamine reaction of DNA deoxypentose(Disch, 1930), where salmon DNA was used as a standard. RNA was estimated by substraction of DNA content from total nucleic acid content.

Determination of soluble carbohydrates

The lyophilized sample was suspended in distilled water (0.1%, w/v) and the homogenate was centrifuged at 20,000 x g for 30 min. The total soluble sugar in the supernatant obtained was determined spectrophotometrically by use of phenol-sulfuric acid reaction(Dubois et al., 1956), where glucose was used as a reference standard.

Determination of starch

500mg of the lyophilized sample were homogenized with an appropriate amount of 80% ethanol and centrifuged to remove the soluble sugar in the supernatant. 52% perchloric acid was added to the residue to dissolve starch and centrifuged, and to resulting supernatant was added then sodium chloride and iodine-potassium iodide solutions to form starch-iodide complex (Colowick and Kaplan, 1957). The starch-iodide complex was precipitated by centrifugation and alcoholic sodium hydroxide was added to the precipitate to liberate the starch by reduction of iodine. The precipitated starch by centrifugation was suspended in 0.7N HCl and transferred to the flask fitted with reflux condensor and heated on the steam bath for 10

hours to hydrolyze the starch. The liberated glucose was determined by use of phenol-sulfuric acid reaction (Dubois et al., 1956).

Determination of total free amino acids

The lyophilized sample was suspended in distilled water (0.1%, w/v) and centrifuged at 20,000 x g for 30 min, and the supernatant obtained was deproteinized with sodium tungstate and dil. sulfuric acid. The free amino acid in the protein-free filtrate was determined by 1-fluro-2,4-dinitrobenzene reaction(Robert, 1963), where glycine was used as a reference standard.

Extraction and determination of total lipids

Lipid was extracted with ether from 5 gm of lyophilized samples in Soxhlet apparatus for 48 hours by heating ether solution to be siphoned every 5 min. The solution was evaporated under reduced pressure and the residue was dissolved in petroleum ether. The solution was filtered through Whatman filter paper No.1 and the filtrate was transferred to the weighing bottle. After the petroleum ether was completely evaporated under reduced pressure, the weight of the total lipids was measured by an analytical balance, and benzene was then added to the bottle to prepare benzene solution for later use in thin-layer chromatography.

Thin-layer chromatography (TLC)

Thin-layer plate 200 x 200 mm, with layer of 0.3mm of Silica gel G(Merck) was prepared in usual way and the plate was activated by heating in an oven at 110°C for 60 min, then cooling in a desiccator. After the sample was applied on the plate with Hamilton microsyringe, the thin-layer chromatogram was developed at room temperature in an ascending direction with a mixture of petroleum ether-diethyl ether-acetic acid, 90:10:1 (v/v). After deveopment, spots in the chromatogram were detected by spraying ammonium molybdate-perchloric acid (Lowenstein, 1969), then heating in an oven at 80°C for color development. The lipid class in the spots was identified by comparison with

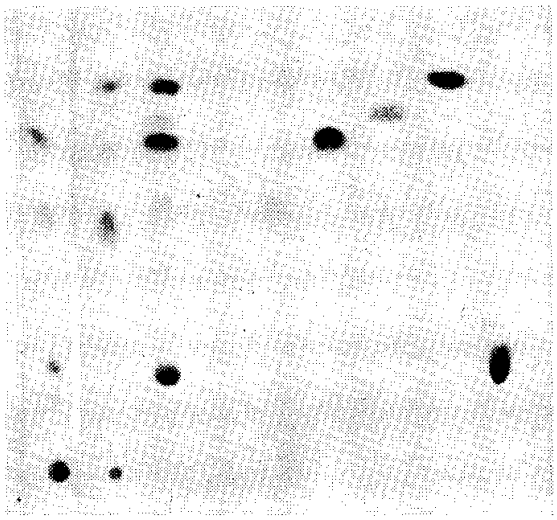

Fig. 1. Separation of pollen lipids and lipid reference compounds applied individually or as a mixture.
1. Lipid from *Ginko*. 2. Lipid from *Pinus rigida*
3. Mixture of referance compounds 4-8,
4. Stearic acid 5. Tristearin 6. Methyl stearate.
7. Cholesteryl stearate 8. Cholesterol

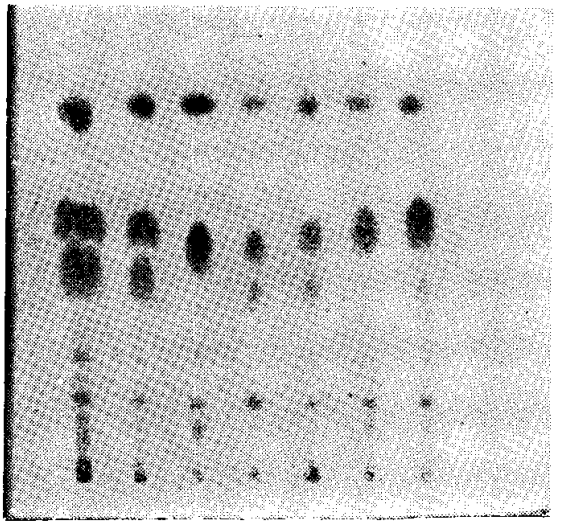

Fig. 2. Separation of lipids by thin layer chromatography from total lipid extracts of pollen species.
1. *Pinus rigida* 2. *Alnus japonica*
3. *Ginko* 4. *Pinus koraiensis*
5. *Pinus aurescens* 6. *Pinus thunbergii*

Rf value of reference compounds such as tristearin for triglyceride, methyl stearate for each fatty acid ester, stearic acid for free fatty acid and, cholesteryl stearate and free cholesterol for bound sterol and free sterol, respectively.(Fig.2)

Quantitative analysis of lipid by TLC

For the quantative analysis of lipid, the thin-layer plate was marked into 6 lanes, 3 cm interval. The samples were applied on the lanes ajacent to 2 outmost lanes rerserved for references and two inner lanes were left for blank. After the plate was developed with the solvent, all the lanes except the outside reference lanes were covered with aluminum foil. The outside reference lanes were sprayed with ammonium molybdate-perchloric acid and the lipid spots on the lane were detected by heating in a vacuum oven at 80°C. The zone corresponding to the lipid spot was marked in the unexposed lane. This zone, together with a corresponding area from the blank lane where no sample was applied, was carefully scraped from the plate onto square of cellophane paper, and transferred to 10 ml glass-stoppered tube for analysis. To each of the tube was added 2 ml of dichromate reagent, prepared by dissolving 2.5g of $K_2Cr_2O_7$ in 1 liter of conc. H_2SO_4. The tube was heated in a water bath at 100°C for 45 min with occasional shaking to bring the silica gel to fine supsension. After the tube was cooled and centrifuged at high speed, the supernatant was ten-fold diluted. Absorbance of the diluted solution was measured at 350 nm against a distilled water blank. The amount of lipid in the unknown mixture was estimated by calculation according to the formula:

$$\frac{\text{Absorbance of unknown} - \text{Absorbance of silica gel blank}}{\text{Absorbance of standard} - \text{Absorbance of silica gel blank}} = \frac{\text{Microgram of lipid in unknown}}{\text{Microgram of lipid in standard}}$$

Preparation of fatty acid esters

The benzene solution of lipid for TLC was evaporated to dryness under reduced pressure and the residue was dissolved in 0.5N alcoholic KOH. The solution was heated in a water bath for 30 min to saponify the lipid. The solution was replenished with alcohol occasionally to prevent the solution from boiling down. After saponification, the solution was evaporated to dryness under reduced pressure and the residue was dissolved in distilled water. Ether was added to the aqueous solution to extract unsaponifiable cholesterol in ether layer. Then the separated aqueous layer was acidified with dil. H_2SO_4 and fatty acid was extracted 3 times with ether from the acidified solution. A few milligrams of N-nitroso-N-methyl urea were added to the combined ether extract in a test tube, then conc. NaOH was added slowly through the wall of the test tube to liberate diazomethane which converts the free fatty acid to their methyl ester. The ether layer was washed with distilled water repeatedly until the aqueous layer was neutral to the phenolphthalein. The ether solution was evaporated and the residue was redissolved in dichloromethane for gas chromatography.

Gas chromatography

Gas chromatography of methyl esters of fatty acid was performed with Pye Unicam GCV equipped with a flame ionization detecter. The glass column (4mm×1.5m) was packed with 10% DEGS on 80—100 mesh Diatomite. The column temperature was 175°C. Nitrogen was used as a carrier gas at flow rate of 30ml/min. The retention times of the reference compounds such as methyl esters of lauric, myristic, palmitic, palmitoleic, stearic, oleic, linoleic, linolenic, and arachidic acid were measured. The individul fatty acid on the gas chromatogram of the sample was identified by those retention

times, but methyl esters of behenic acid was identified from the straight line obtained by potting $\log_{10}$ retention time versus the number of carbon atom in the saturated fatty acid chain (Fig. 3). The composition of fatty acid was calculated by measuring the individual peak area by triangulation procedure.

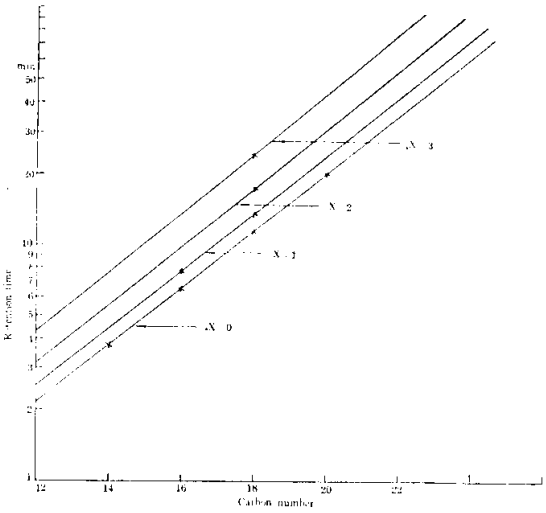

Fig 3. Plot of retention time for standard fatty acid methyl esters on semilogarithmic paper.

RESULTS AND DISCUSSION

Results expressed as percent of dry weight material were means of triplicate or duplicate analyses on each aliquot. The maximum fluctuation between each analysis was less than 5% of mean value.

As shown in Table 1, the nucleic acid content of pollen examined does not vary widely except gingko, showing around 0.05% for DNA and 0.72-1.02% for RNA in *Pinaceae*. The gingko pollen contains, however, about three times as much DNA as *Pinus* species, thus confirming the result of our previous report(Lee and Chung, 1976)

The amount of chromosomal DNA in the nuclei of different species of higher plants varies enomously and all are diploids with the same

Table 1. Pollen nucleic acid contents

Pollen Species	% Dry Weight		
	Total nucleic acid	DNA	RNA
Gingko biloba L.	3.87	0.14	3.73
Pinus rigida	0.86	0.05	0.81
Pinus koraiensis	0.83	0.05	0.78
Pinus thunbergii	1.02	0.05	0.97
Pinus aurescens	0.85	0.05	0.80
Pinus densiflora	1.08	0.06	1.02
Aluns japonica	0.75	0.03	0.72

number of chromosomes(2n=14), although the chromosome size varies considerably. But chromosome size is closely correlated with nuclear DNA content(Rees, 1972). Flavell et al.(1974) also reported that the 2 C nuclear DNA contents of the species varies between 1.7 and 98 pg.

The chromosome number of gingko and pine (densiflora) is 16 and 24 respectively in diploid cell(Ahn, 1963). Thus, pine is superior to gingko in chromosome number. The ploidy of pollens concerned was neither determined nor found in available references. In connection with the pollen DNA content it should be recalled that the mature pollen grains contain either two or three nuclei, that is, a vegetative nucleus and either a generative nucleus or two sperm nuclei which arise from mitotic division of a generative nucleus. But the pollen size of gingko was shown about 3 times as much mass as that of Pinus. The higher DNA content in gingko pollen is supposedly attributable in part to pollen size, comparing with *Pinus*, because of the cell weight or size being roughly parallel to DNA content(Flavell et al.,1974).

On the other hand RNA comprises about 0.72−1.02% of pollen dry weight except gingko (3.73%), indicating much low content, in comparison with the former data (Lee and Chung, 1976). It seems to be due to the application of different method for estimation of RNA. The

RNA content was previously determined by orcinol reaction of ribose but this time it was estimated indirectly by the difference between total nucleic acid and DNA contents, because the extreme abundance of carbohydrates in plant material interferes much with the orcinol reaction(Ingle, 1963).

Togasawa et al. (1968) reported that in *Pinus densiflora*, DNA and RNA occur 0.016% and 0.14% respectively. Stanly and Yee (1966) found one tenth lower percent of DNA(0.05 %) and RNA(0.5%) in *Pinus ponderosa* than Euler et al.(1948) found in *Pinus montana*. While this may possibly be attributed to a larger mass of enclosing wing and exine in *P. ponderosa*, it seems more likely to be the result of improved assay procedure. Thus the DNA value of *Pinaceae* in present paper is just same as that of Stanly and Yee (1966). but is still higher than that of Togasawa et al.(1968). In general, pre-1960 literature reported DNA content at level of about 0.5% of the dry weight and total RNA content varying from 0.6% to 10%. More accurate assay procedure with less contamination should be exploited for the determination of nucleic acid in plant materials.

Pollen grains contain 11-16% of the total soluble carbohydrate on dry weight basis(Table 2), which seem to be used as principal metabolic substrate upon germination of pollen. The level varies with species, harbitat, and harvesting and storage conditions. Fructose, glucose and sucrose are the free sugars in highest concentration in ethanol extracts of pollens(Motomura et al., 1962).

Table 2. Soluble carbohydrate and starch contents of pollens

Pollen Species	% Dry weight	
	Water-soluble carbohydrate as glucose equivalent	Starch
Gingko biloba L.	11.4	—
Pinus rigida	15.0	2.82
Pinus koraiensis	16.2	7.06
Pinus thunbergii	12.5	3.53
Pinus aurescens	11.6	0.59
Pinus densiflora	12.3	2.54
Alnus japonica	13.8	1.62

As shown in Table 2, starch content of *Pinus* pollen varies with narrow range of 2.5-3.5% in 3 species(*P. densiflora*, *P. rigida and P. thunbergii*), but occurs as much as 7.0% in *P. koraiensis* and as low as 0.6% in *P.aurescens*. Alnus pollen contains 2.8% of starch. It is noteworthy that the starch was absent in gingko pollen in repeating analysis, in contrast to very high content of *P. koraiensis*. Huegel (1965) reported that starch content of pollen is highly variable, comprising as much as 12.4% of the dry weight of *Typhalatifolia* down to 2.6% in *P. thunbergii* which is well comparable with ours. The wide variation in starch content may be in part due to environmental factors (temperature, duration, humidity) upon storage which could cause pollens to carry out oxidative metabolism. Thus, function of carbohydrate storage in pollen could be understood in connection with viability of pollens. Apart from animal sperm cells, pollen grains have been proved

Table 3. Free amino acid contents of pollens

Pollen Species	*Gingko biloba* L.	*Pinus rigida*	*Pinus koraiensis*	*Pinus thunbergii*	*Pinus aurescens*	*Pinus densiflora*	*Alnus japonica*
% Dry weight as nitrogen of free amino acids	0.90	0.38	0.38	0.43	0.42	0.51	0.64

to survive upon prolonged storage, though depending on the species.

The free amino acid contents of pollen range from 0.38% to 0.51% in 5 species of *Pinus*, while scores 0.9% and 0.64% in gingko and *Alnus japonica* respectively(Table 3). Analytical data of the free amino acids in pollens have been reported (Linskens and Schrauwen, 1969) and it was shown that all the essential amino acids are present in pollens and the free amino acids do not necessarily reflect the mole ratio of amino acids in pollen protein. The free amino acid contents can vary with the climate and nutritional conditions of plants on which the pollen matures (Tseluiko, 1968). It seems likely that high level of amino acids together with soluble carbohydrates is very important in keeping pollen grains viable upon storage. Because of the importance of pollen proteins in allergy response and the nutirtional interest, the analysis of pollen proteins is now undertaken in this laboratory as well as pollen free amino acid pattern and amino acid composition of pollen proteins.

As shown in Table 4, the total lipid content of pollen varies from 3.87% to 6.28% in species of *Pinus* and 2.39% and 3.46% are accounting for gingko and *Alnus japonica*, respectively. And the triglyceride contents of pollens examined range from 1.09% to 2.23%. Ether extracts of pollen variy considerably and the exine-contained lipid material is probably the major source of variation in ether-extractable materials. The comparison of fat and starch contents of different pollen was the basis for the classification by Calvins(1974) of 1,170 species, she correlated the storage energy source with dispersal mechaniam, i.e. species high in fat are distributed by bees, while pollen high in starch are wind-dispersed. Generally, the percent lipids will run 5% or less.

The free fatty acid contents vary from 0.13

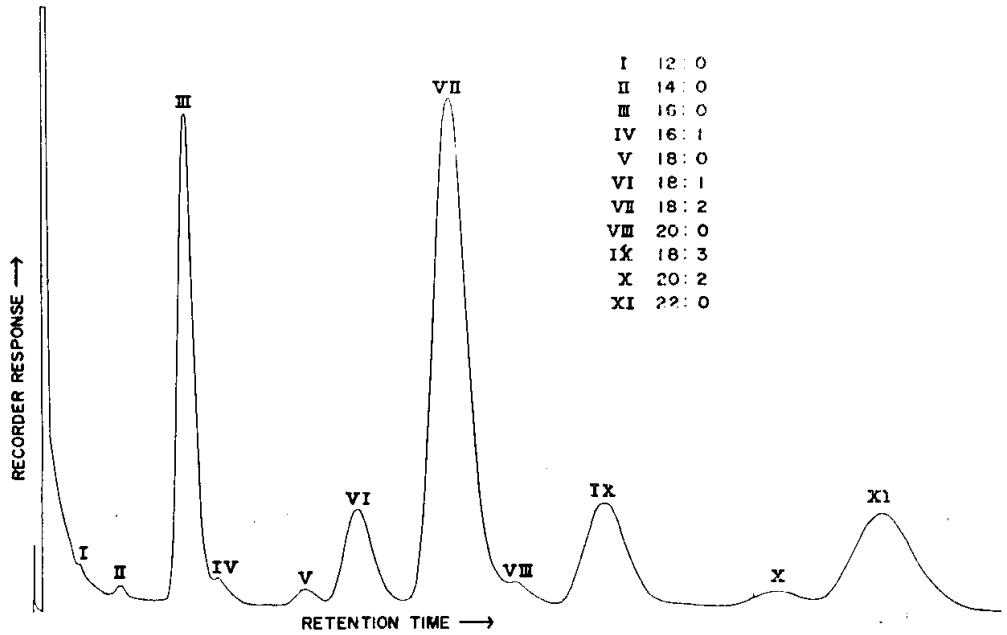

Fig. 4. Gas chromatogram of methyl esters of fatty acids in the saponifiable fraction of lipid from gingko pollen on 1.5m×4mm (I. D.) glass column packed with 10% DEGS on 80-100 mesh Diatomite; temperature 175°C; nitrogen carrier gas 30ml/min; flame ionization detector; attenuation 8×10^2.

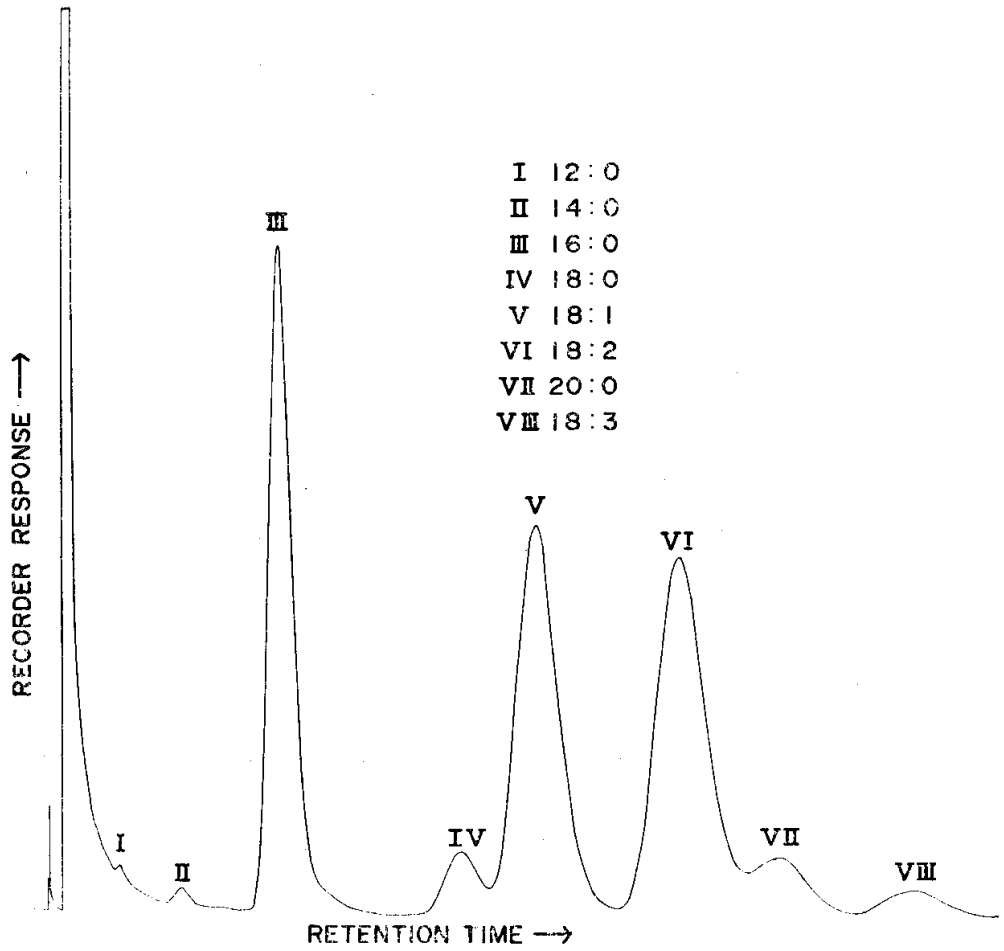

Fig. 5. Gas chromatogram of methyl esters of fatty acids in the saponifiable fraction of lipid from *P. koraiensis* pollen on 1.5m×4mm (I.D.) glass column packed with 10% DEGS on 80-100 mesh Diatomite; temperature 175° C; nitrogen carrier gas 30ml/min; flame ionization detector; attenuation 8×10^2.

% to 2.0% in *Pinus* species, which 0.26% in gingko and very low value of 0.06% in *Alnus japonica*(Table 4). Ching(1962) reported 1.25—1.33% dry weight free fatty acids in 2 species of freshly harvested pinus pollens.

The fatty acids are primarily recovered in the saponified portion of lipid extract. Table 5 shows the distribution of fatty acids in saponifiable fractions of the pollen lipids. Eight fatty acids in both genera *Pinaceae* and *Alnus* and 11 in gingko were detected in the gas chromatogram(Fig. 4, 5), though 7-10 fatty acids were identified. The major fatty acids are palmitic acid(25.0—28.1%), oleic acid(29.8—41.5%) and linoleic acids (24.9—33.5%) in *Pinus* species, while palmitic acid 19.3%, linoleic acid 42.6%, linolenic acid 11%, behenic acid 14% in gingko, and palmitic acid 11.6%. oleic acid 16.5%, linoleic acid 43.4%, linolenic acid 26.8% in *Alnus japonica*. In general, very low palmitic acid value is scored in all the pollens examined, and arachidic acid (3.7%-5.2%) occurs only in *Pinus* species. It is noticeable that much higher linolenic and lower oleic

Table 4. Distribution of pollen lipids

Pollen Species	Total lipid	Triglyceride (% dry weight)	Free fatty acid	Sterol ester	Sterol	Sterol-ester / free sterol
Gingko biloba L.	2.39	1.09	0.26	0.30	0.20	1.5
Pinus rigida	6.28	1.88	2.00	1.66	0.13	12.7
Pinus koraiensis	5.84	1.72	1.10	1.09	0.12	9.0
Pinus thunbergii	4.68	2.23	0.54	0.67	0.09	7.4
Pinus aurescens	3.87	1.69	0.13	0.59	0.16	3.7
Alnus japonica	3.46	1.48	0.07	0.59	0.10	5.9

Table 5. Distribution of fatty acid components in saponifiable fraction of pollen lipids

Pollen Species	Fatty acids (% weight as methyl esters)										
	Lauric	Myristic	Palmitic	Palmi-toleic	Stearic	Oleic	Linoleic	Linolenic	Ara-chidic	Behenic	
	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	22:0	20:2
Gingko biloba	trace	0.5	19.3	1.3	1.1	7.3	42.6	11.1	trace	14.6	2.2
Pinus rigida		trace	28.1		1.2	33.5	33.5	trace	3.7		
Pinus koraiensis		trace	27.9		3.4	29.8	31.8	2.1	5.0		
Pinus thunbergii		trace	25.3		2.0	40.7	26.6	1.4	4.0		
Pinus aurescens		0.5	25.0		2.9	41.5	24.9		5.2		
Alnus japonica		0.4	11.6		1.3	16.5	43.4	26.8			

acids are contained in gingko pollen, compared with those of *Pinus*, and the occurrence of behenic acid only in gingko pollen. While very high linolenic acid level is found with lower palmitic acid in *Alnus japonica*. Similar fatty acid pattern and scanty amount of trienoic acid are also characteristic in *Pinus* pollens.

Ching and Ching(1962). applying gas chromatographic analysis, detected 16 different fatty acids in 5 species of *Pinus*, reporting that palmitic and oleic acids were major components, e.g. 14 to 31% of the fatty acids extracted from *P·ponderosa* and *P. contota* pollens, agreeing closely with the present data.

The bound and free sterol contents of pollens vary 0.3—1.66% and 0.09—0.2%, respectively and the ratio of bound sterol to free one ranges from 1.5% to 12.7%. Thus, the pollen sterol content varies considerably from species to species with more bound form than free one.(Table 4).

The amounts and types of sterols vary between different pollen species. Pinus pinaster pollen extracts contained more bound sterols than were free(Dungworth et al.,1971). The general conclusion of Standifer et al.(1968) based on data from 15 pollen species, is that no simple phylogenetic relationship is discernable from the sterol data.

SUMMARY

An attempt was made to reassess the nucleic acid contents of pollen materials in this paper, dealing with 6 species of gymnosperm, including

5 species of *Pinus* and one *Gingko* species, and one *Alnus* species of angiosperm as comparison. The starch, water soluble sugar and free amino acid patterns in pollens were examined as well as their lipid compositions and fatty acid patterns of saponified lipids.

Results obtained are summarized as follows:

1. The DNA content was around 0.05% on dry weight basis of pollen in 5 species of *Pinus*. *Gingko* pollen, however, contained about 3 times as much DNA as in *Pinus* and *Alnus japonica* 0.3%. The discussion was made in relation to the higher DNA content in *Gingko* pollen. The RNA content comprised ranging from 0.8% to 1.02% in 5 species of *Pinus*, while 3.73% in gingko and 0.72% in *A. japonica*, indicating exceptionally high RNA content in *Gingko* pollen.

2. The soluble carbohydrate content in pollen ranged from 11.4% to 16.2%. The pollen starch content varied with a narrow range of 2.5—3.5% in 3 species of *Pinaceae*, but as much as 7.0% in *P. koraiensis* and as low as low as 0.6 % in *P. aurescens*. It is noticeable that no starch was detectable in *Gingko* pollen.

3. The nitrogen content of free amino acids of pollens comprised 0.38% to 0.51% in 5 species of *Pinaceae*, while 0.97% and 0.64% in *Gingko* and *A. japonica*, respectively.

4. The total lipid content was in the range of 3.87% to 6.28% in *Pinus* pollens and 2.39 % in *Gingko* and *A. japonica*.. The free fatty acid content was variable from 0.13% to 2.0% in *Pinus* and 0.26% in *Gingko* and as low as 0.06% in *A. japonica*. The bound and free sterol content of pollens varied considerably 0.3—1.66% and 0.09—0.2%, respectively and the ratio of bound sterol to free one ranged from 1.5% to 12.7%, indicating more bound form than free one.

5. The gas chromatograms detected 8 fatty acids in both genera *Pinus* and *Alnus* and 11 ones in *Gingko*, though 7 to 10 fatty acids were identified. The major fatty acids are palmitic acid(25.0—28.1%), oleic acid(29.8-41,5%) and linoleic acid (24.9—33.5%) in *Pinus* species, while palmitic acid 19.3%, linoleic acid 42.6%, linolenic acid 11%, behenic acid 14% in *Gingko*, and palmitic acid 11.6%, oleic acid 16.5%, linoleic acid 43.4%, linolenic acid 26.8% in *Alnus japonica*. It is noteworthy that much higher linolenic acid was contained in *Gingko* pollen with lower oleic acid, as compared with those of *Pinaceae*, and behenic acid occurred only in *Gingko*.

》국문초록《

Gymnosperm 花粉의 화학성분에 관한 연구

서울대학교 의과대학 생화학교실

정 홍 근·이 기 녕

Gymnosperm에 속하는 5종의 소나무 화분과 은행화분및 비교로서 angiosperm에 속하는 오리나무화분에서 DNA, RNA, 전분, 수용성당질, 유리아미노산 및 지질의 함량과 총지질중의 지방산의 분포에 관한 연구 결과의 요지는 다음과 같다.

1. DNA함량은 건조된 화분의 중량을 기준으로 하여 5종의 소나무화분에서는 대략 0.05%이지만 은행화분에서는 소나무화분 함량보다 대략 3배 정도 높고 오리나무 화분에서는 0.03%로서 가장 낮다. RNA 함량은 DNA 함량보다 대략 10배 정도 높으며 은행화분에서 가장 높아서 3.73%나 된다.

2. 화분의 수용성 당질의 함량은 11.4~16.2%로서 화분의 종류에 따라 큰 변화가 없다. 그러나 전분의 함량은 화분의 종류에 따라 변화가 커서 잣나무화분에서는 7.0%로서 가장높고 은행화분에서는 전분이 검출되지 않았다.

3. 유리 아미노산의 질소함량은 은행화분에서 가장 높아 0.97%이고 나머지에서는 0.38~0.64%이다.

4. 총지질의 함량은 대략 2.4~6.3%이고 총지질을 TLC로 분리한 결과는 triglyceride(1.09~2.23%) 및 sterol ester (0.30~1.66%)가 주성분이며 sterol은 free sterol보다는 주로 sterol ester로 존재함을 보여준다. 그리고 유리지방산함량은 특히 잣(1.1%) 및 리기다소

나무(2.0%)에서 높음을 보여준다.

5. 총지질을 검화하여 얻은 지방산을 가스크로마토그라피법으로 분석한 결과는 소나무화분에서는 palmitic (25.0~28.1%), oleic acid(29.8~41.5%) 및 linoleic acid (24.9~33.5%)가 주성분임을 보여주고 오리나무 및 은행에서는 불포화도가 더높은 linolenic acid가 각각 26.8% 및 11.1%로 존재하며 소나무에서 소량으로 검출되는 arachidic acid는 검출되지 않았다. 은행화분에는 다른 화분에서 검출되지 않는 behenic acid(14.6%)가 비교적 많은 양으로 존재하여있다.

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