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HYDROCARBONS AND FATTY ACIDS OF *LYCOPODIUM*

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Key Word Index—*Lycopodium*; Lycopodiaceae; fatty acids; alkanes; alkenes; isoprenoids; organic geochemistry.

Abstract—The analysis of fatty acids and hydrocarbons in the sporophytes of three *Lycopodium* species has revealed a characteristic distribution of C_{16} and C_{18} acids. The hydrocarbon fraction of the lipids contain a homologous series of monounsaturated alkenes in the C_{17}–C_{30} range with an even to odd preference. Maxima at both C_{17} and C_{27} among the *n*-alkanes reveals similarities both to the distribution of hydrocarbons in other plant groups. The production of spores and their inclusion with one sporophyte does not alter the fatty acid pattern but does decrease the alkene concentration and modifies the alkane distribution, shifting both maxima. The presence of pristane and phytane in all specimens, the dual maxima of alkanes and slight odd to even preference of alkanes is noteworthy in that these characteristics are possessed by geological deposits derived from *Lycopodium* ancestors.

INTRODUCTION

THOUGH relegated to the low-creeping evergreen of the woodlands, *Lycopodium* belongs to the same general line of development as did the *Lepidodendron* of the Carboniferous Era; this arborescent ancestor, dominated many of the forests of that era.[1] This present day 'remnant of the Coal Age' represents a group of plants positioned in phylogenetic trees beneath the ferns and flowering plants and above the algae, fungi, lichen, liverworts and mosses. Because of its supposed ancient lineage and intermediate phyletic position, *Lycopodium* was chosen to serve as a possible link between 'higher' and 'lower' plants as well as a key to the biosynthetic processes prevalent in the past.

The objective in undertaking the chemical characterization of *Lycopodium alopecuroides* L., *L. adpressum* (Chapm.) Lloyd and Underw. and *L. carolinianum* L.,[2] was to compare the results with those from other members of the plant kingdom and interpretive comparison with geologically preserved organic matter based on earlier plant forms. The comparative biochemistry of lignins and sugars in *Lycopodium* has been reported,[3] and Swain *et al.*[4] have related the carbohydrates in some fossil lycopods and modern pteridophytes to evolutionary pathways. Since hydrocarbons and fatty acids are of special value in

[1] H. N. WHITE, *Ancient Plants and the World They Lived In*, Comstock, Ithaca (1964).
[2] A. E. RADFORD, H. E. AHLES and C. R. BELL, *Manual of the Vascular Flora of the Carolinas*, The University of North Carolina Press, Chapel Hill (1968).
[3] E. WHITE and G. H. N. TOWERS, *Phytochem.* **6**, 663 (1967).
[4] F. M. SWAIN, J. M. BRATT and S. KIRKWOOD, *J. Paleontol.* **42**, 1078 (1968).

such evolutionary studies,[5] we chose to examine these substances in the above *Lycopodium* species.

TABLE 1. HYDROCARBON AND FATTY ACID ANALYSIS OF *Lycopodium* SPOROPHYTES

Percentage composition	*L. alopecuroides* No sporulation	*L. alopecuroides* In sporulation	*L. adpressum* In sporulation	*L. carolinianum* In sporulation
Lipid/dry wt	8·01	8·24	5·58	5·43
HC/dry wt*	0·008	0·01	0·001	0·006
Fatty acid/† dry wt	2·95	2·67	0·25	4·43
HC/lipid	0·10	0·14	0·02	0·12
Fatty acid/lipid	36·82	32·43	4·41	81·49

The sporophytes were collected with and without spores for each species as indicated. Specimens without spores were taken prior to sporulation.

* HC is the weight of the hexane eluate of combined silica gel-alumina chromatography of total lipids.

† Fatty acid weight is weight of total fatty acid fraction as methyl esters.

RESULTS

The three *Lycopodium* species exhibited little variation in their lipid weights. Table 1 indicates that the lipid weight constitutes 5–8% of the total dry plant wt. In this complex mixture of lipids the fatty acids comprise by far the largest fraction. *Lycopodium alopecuroides* and *L. carolinianum* contain between 2·7 and 4·4% (dry plant wt) fatty acids whereas *L. adpressum* contains but 0·25% fatty acids; three replicate extractions failed to yield higher results for this species. More than 98% of the fatty acids resided in the C_{16}–C_{18} region with palmitic or oleic acid predominating. Though present at considerably lower levels of concentration, the higher carbon-number fatty acids, presumed as the precursors to the higher hydrocarbons,[6] were also characterized. Treating the metabolic (C_{14}–C_{18}) acids and the higher acids as two distinct entities, each identified fatty acid and the relative percentage within its respective group is listed in Table 2 or displayed in Fig. 1. Included in Fig. 1 are the unsaturated fatty acids, which are mostly monounsaturated with lesser quantities of the di- and poly-unsaturated acids. The reported fatty acids, ranging from carbon number 14–30 account for *ca.* 99% of those detectable by GLC.

TABLE 2. METABOLIC FATTY ACIDS OF *Lycopodium* SPECIES IN SPORULATION

Fatty acid	*L. alopecuroides*	*L. adpressum*	*L. carolinianum*
$C_{14:0}$*	2·36	2·51	0·63
$C_{16:0}$ (palmitic)	22·67	58·01	20·24
$C_{16:1}$	tr	tr	tr
$C_{18:0}$	tr	2·08	tr
$C_{18:1}$ (oleic)†	59·79	22·90	72·41
$C_{18:2}$	15·18	12·73	6·72
$C_{18:3}$	tr	—	tr

Fatty acids expressed as % (GLC peak area of component/(GLC peak area of total F. acids).

* $C_{n:x}$ (n—No. of carbon atoms: x—No. of double bonds).

† Corresponds to oleic acid by R_f and by coinjection.

[5] G. EGINTON and R. J. HAMILTON, in *Chemical Plant Taxonomy* (edited by T. SWAIN), p. 187, Academic Press, London (1963).

[6] G. EGLINTON and R. J. HAMILTON, *Science* **156**, 1322 (1967).

The hydrocarbon fraction of the lipids constitutes a very small percentage of the lipids, but though limited in concentration, extreme complexity was observed in the component distribution (Fig. 2). The normal alkanes fell into two groups centered around C_{17} or C_{18} and C_{25}, C_{27} or C_{29}. The higher MW portion of the hydrocarbon profile dominated, but C_{17} or C_{18} occurred in concentrations exceeded only by a few of the hydrocarbons of higher MW. Table 3 indicates a slight predominance of the odd numbered *n*-alkanes with a CPI value[7] of *ca.* 2·5. A homologous series of normal monounsaturated alkenes represented between 10 and 25% of the total hydrocarbon fraction and occurred in both the spore producing and pre-spore producing stages of *L. alopecuroides*. Among the normal alkenes the predominance of odd over even was reversed (see Fig. 2).

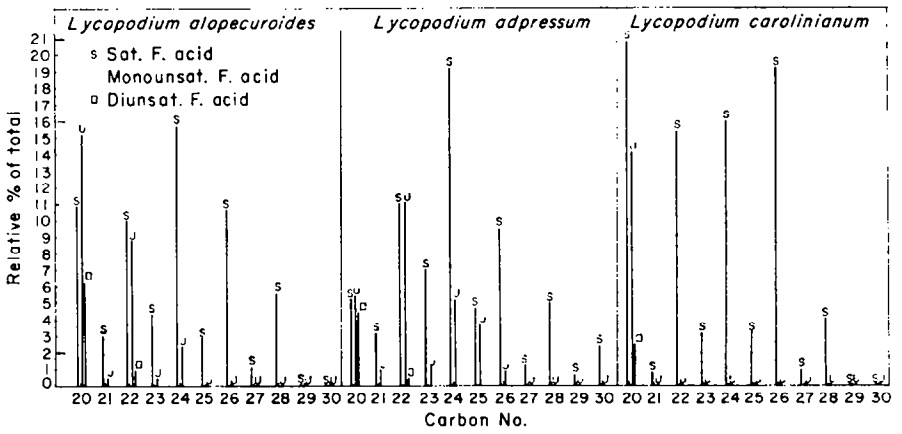

FIG. 1. HIGH MW FATTY ACIDS IN *Lycopodium* SPP. SPOROPHYTES.
The normal saturated, monounsaturated and diunsaturated fatty acids of the sporophytes of three *Lycopodium* spp. are shown with the corresponding relative % (computed by GLC peak volume) of the C_{20}–C_{30} fatty acid fraction.

In the lower MW region, the saturated isoprenoids, pristane and phytane were identified in concentrations totaling 2–5% of the total hydrocarbon fraction. Other isoprenoids were evidenced by the substantial increase of phytane and pristane concentrations in a fully saturated fraction of the hydrocarbons. GLC data revealed at most only trace amounts of the *iso*- and *anteiso*-branched hydrocarbons. The remainder of the hydrocarbons (8–30%) were composed of from one to several polyunsaturated, branched hydrocarbons in the C_{19}–C_{24} carbon range. The type of compound as well as concentrations varied from one species to another. One such hydrocarbon occurring as a major component of the pre-spore producing stage of *L. alopecuroides* was only a very minor component of the spore containing plant. These 'specialized' hydrocarbons deserve further attention.

DISCUSSION

The strong predominance of oleic acid in *L. alopecuroides* and *L. carolinianum* can be seen in Table 2. *Lycopodium adpressum*, however, shows an equally strong predominance for palmitic acid. Virtually no variation was noted in the fatty acids in *L. alopecuroides* collected

[7] J. E. COOPER and E. E. BRAY, *Geochim. Cosmochim. Acta* **27**, 1113 (1963).

prior and during sporulation; therefore, comparisons to leaf lipids of other plant groups[8–10] is probably more appropriate. It is doubtful that the spores included in three of the samples contributed much to the total fatty acid content, for in *L. clavatum*[11,12] and *L. volubile*[13] the dominant fatty acid of the spore lipids (up to 35% of total lipid wt) was the $C_{16:1}$ acid, which is only a minor component in the present species studied. The distribution of fatty acids among these three species bears only a very general similarity to the distributions found among other plants.

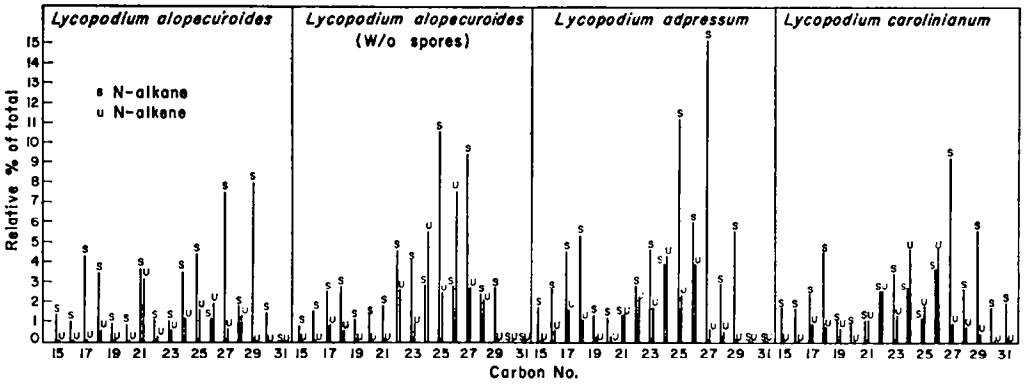

FIG. 2. ALKANES AND ALKENES OF *Lycopodium* SPP. SPOROPHYTES
The normal hydrocarbons of the total plant of three *Lycopodium* spp. including a pre-spore sporophyte of *L. alopecuroides* are depicted as their relative % of the total hydrocarbon fraction assuming that the GLC data represents 100% of the hydrocarbons. Pristane, phytane and other isoprenoidal compounds comprise the remainder of the hydrocarbon fractions.

Reports of the higher fatty acids are exceedingly rare; as Fig. 1 demonstrates, significant quantities of the C_{20} acids do occur in the higher MW fraction of these *Lycopodium* species. An interesting feature is the assemblage of higher monounsaturated fatty acids ranging from $C_{20:1}$ to $C_{26:1}$. As in the saturated fatty acids, there is a decided even to odd preference. *Lycopodium carolinianum* is exceptional in that only the C_{20} homologue occurs in measurable concentrations.

The alkane distribution of the *Lycopodium* species displayed evinces remarkable similarities to other plant groups.[14–18] The maxima at C_{17} or C_{18} and at C_{25}, C_{27} or C_{29} and a CPI value of at most 2·67 (Table 3) reflect qualities somewhat intermediate to plants phylogenetically more or less advanced than *Lycopodium*.

[8] R. G. ACKMAN, C. S. TOCHER and J. MCLACHLAN, *J. Fish. Res. Bd. Can.* **25**, 1603 (1968).
[9] P. M. WILLIAMS, *J. Fish. Res. Bd. Can.* **22**, 1107 (1965).
[10] F. B. SHORELAND, in *Chemical Plant Taxonomy* (edited by T. SWAIN), p. 253, Academic Press, London (1963).
[11] J. L. RIEBSOMER and J. R. JOHNSON, *J. Am. Chem. Soc.* **55**, 3352 (1933).
[12] H. WAGNER and H. FRIEDRICH, *Naturwissenschaften* **52**, 305 (1965).
[13] I. M. MORICE, *J. Sci. Food Agric.* **13**, 666 (1962).
[14] A. G. DOUGLAS and G. EGLINTON, in *Comparative Phytochemistry* (edited by T. SWAIN), p. 57, Academic Press, London (1966).
[15] M. BLUMER, R. R. L. GUILLARD and T. CHASE, *Mar. Biol.* **8**, 183 (1971).
[16] W. W. YOUNGBLOOD, M. BLUMER, R. L. GUILLARD and F. FIORE, *Mar. Biol.* **8**, 190 (1971).
[17] E. GELPI, J. ORO, H. J. SCHNEIDER and E. O. BENNETT, *Science* **161**, 700 (1968).
[18] R. C. CLARK, JR. and M. BLUMER, *Limnol. Oceanogr.* **12**, 79 (1967).

The presence of a homologous series of normal alkenes in all three species deserves special attention. Though the presence of the *n*-alkenes is not unknown[16,17,19] the even to odd preference of the alkenes is unusual and more pronounced than in most previous analyses of alkenes.[5] The presence of plant alkenes has been explained as either metabolic intermediates in the biosynthesis of alkanes[18] or as dehydrogenation products of alkanes or dehydration products of secondary alcohols or other intermediates. If the alkenes are indeed metabolic intermediates, then correlation of the alkanes and alkenes would not be unexpected. Correlation coefficients, therefore, were calculated to test the validity of this assumption. Treating each plant separately, coefficients were calculated for the following pairs of hydrocarbon variables, C_n (alkanes) vs. $C_{n:1}$ (monounsaturated alkenes), C_n vs. $C_{(n+1):1}$ and C_n vs. $C_{(n-1):1}$, $n = 15,16, \ldots 31$. High correlation at the 99% confidence level was observed only for C_n vs. $C_{(n-1):1}$, for all samples but the *Lycopodium alopecuroides* with spores (probably due to the poor resolution of certain GLC peaks in this one sample). The high correlation of the alkenes to alkanes of one higher carbon number would suggest a biosynthetic pathway involving a chain lengthening of the alkenes in the production of alkanes.

TABLE 3. HYDROCARBON COMPONENTS OF *Lycopodium* SPOROPHYTES

Component	*L. alopecuroides* No sporulation	*L. alopecuroides* In sporulation	*L. adpressum* In sporulation	*L. carolinianum* In sporulation
Total *n*-alkanes	50·43	44·41	69·43	58·82
Total *n*-alkenes	24·80	10·78	18·39	20·17
Pristane	1·61	2·88	3·10	1·24
Phytane	1·30	1·34	1·89	1·24
CPI* of alkanes	2·15	2·67	2·35	2·49

Hydrocarbon entries listed as % (GLC peak area of component)/(GLC peak area of all components). The sporophytes were collected with and without spores for each species as indicated. Specimens without spores were taken prior to sporulation.

$$* \text{CPI} = \frac{1}{2}\left[\frac{\sum_{n=21}^{n=31} C_n\text{odd}}{\sum_{n=20}^{n=30} C_n\text{even}} + \frac{\sum_{n=21}^{n=31} C_n\text{odd}}{\sum_{n=22}^{n=32} C_n\text{even}}\right]$$ (see Cooper and Bray[7]).

The occurrence of the isoprenoids, particularly pristane and phytane, in *Lycopodium* is intriguing. At the present time, only pristane has been reported in the hydrocarbon fractions of flowering plants though phytane and especially pristane have been recovered from algal and bacterial lipids.[15,16,18,20,21] Several species of ferns, spleenworts, liverworts and mosses have exhibited GLC R_ts corresponding to pristane and phytane[22,23] so that it appears that the synthesis of these isoprenoids extends at least through the Pteridophyta.

[19] G. A. HERBIN, *E. African Acad. Sci.* 2nd Symposium (1964).
[20] J. R. SEVER, Ph.D. Thesis, The University of Texas at Austin (1970).
[21] J. R. MAXWELL, C. T. DILLINGER and G. EGLINTON, *Q. Rev. Chem. Soc.* **25**, S71 (1971).
[22] J. R. SEVER, T. F. LYTLE and P. HAUG, *Contributions in Marine Science*, Vol. 16, p. 149, University of Texas, Texas (1972).
[23] J. R. SEVER and T. F. LYTLE, unpublished data.

One observes that *L. alopecuroides* undergoes changes in its hydrocarbon distribution with the onset of spore production, i.e. a shift in dominant hydrocarbon from C_{25} in the pre-spore stage to C_{29} and reduced alkene contribution in the spore stage. This makes it difficult to distinguish chemically the three species of *Lycopodium*. However, by considering the spore producing plant as more representative of the total plant, the summary contained in Table 4 will suffice clearly to contrast these *Lycopodium* species.

TABLE 4. PREVALENT FEATURES OF THE HYDROCARBONS AND FATTY ACIDS *Lycopodium* SPP. SPOROPHYTES WITH SPORES

Component	*L. alopecuroides*	*L. adpressum*	*L. carolinianum*
C_{20}–C_{31} alkanes	sl. dominance of C_{29}	dominance of C_{27}	dominance of C_{25}
C_{15}–C_{19} alkanes	sl. dominance of C_{17}	sl. dominance of C_{18}	sl. dominance of C_{18}
Metabolic F. acids	dominance of $C_{18:1}$	dominance of $C_{16:0}$	dominance of $C_{18:1}$
C_{20}–C_{30} F. acids	C_{20}–C_{26} monoenoics	C_{20}–C_{26} monoenoics	C_{20} monoenoic

The presence or (slight) dominance of certain entities are listed which seem to distinquish the three *Lycopodium* spp.

Some of the distinguishing characteristics of the hydrocarbons of *Lycopodium*, viz. the presence of pristane and phytane, dominance of C_{17} or C_{18} and low CPI values may be duplicated in the biochemical profiles preserved in ancient sedimentary organic matter. Indeed these characteristics are present in hydrocarbon distributions of certain coals,[24] oil-rich shales,[25,26] petroleum[27,28] and other ancient sedimentary deposits which are now generally assumed to be reservoirs of ancient plant remains. Since *Lepidodendron*, a closely allied species of *Lycopodium*, was the primary contributor of the coal deposits of southern Illinois,[1] further studies of *Lycopodium* and other pteridophytes and bryophytes could be valuable in interpretive studies of fossil organic matter.

EXPERIMENTAL

Collection and preparation. All three species were collected during spore production on 19 September 1971. The *L. alopecuroides* was also collected just prior to spore production in April 1972. Identification and classification of plants was made by L. N. Eleuterius. Approximately 500 whole plants, including spores, were rinsed with distilled H_2O, air dried, then dried in an oven at 40° for 12 hr, pulverized in a Waring blender and re-dried for 12 hr at 40°. 100 g was extracted three times with $CHCl_3$ using ultrasonic vibrations. The $CHCl_3$ extracts were then taken to near dryness. After transferring the concentrate into a volumetric flask, a 1/20 aliquot was removed for a lipid wt determination. Solvents used in all procedures were reagent grade and glass distilled through reflux columns prior to use. All precautions were taken throughout the procedures to minimize losses and contaminations. Solvent and reagent blanks were prepared to correct for contamination levels which were negligible.

[24] K. KOCHLOEFL, P. SCHNEIDER, R. RERICHA, M. HORAK and V. BAZANT, *Chem. & Ind.* 692 (1963).
[25] G. EGLINTON, P. M. SCOTT, T. BELSKY, AL. L. BURLINGAME and M. CALVIN, *Science* **145**, 263 (1964).
[26] T. BELSKY, R. B. JOHNS, E. D. MCCARTHY, A. L. BURLINGAME, W. RICHTER and M. CALVIN, *Nature, Lond.* **206**, 446 (1965).
[27] J. G. BENDORAITES, B. L. BROWN and L. S. HEPNER, *Analyt. Chem.* **34**, 49 (1962).
[28] R. A. DEAN and E. V. WHITEHEAD, *Tetrahedron Letters* 768 (1961).

Solvolysis of plant lipids. Each lipid sample was saponified by refluxing with 0·5 N KOH–MeOH for 1 hr. Nonsaponifiable components were removed by extraction of the methanolic solution with benzene. Benzene-soluble plant acids were extracted after acidification of the alkaline solution. Methyl esters of the fatty acids were prepared using BF_3–MeOH.

Preparative column chromatography. The nonsaponifiable residue was fractionated on a glass column (43 × 2·5 cm, o.d.) packed with silica gel (activity grade I deactivated with 5% H_2O) beneath 25 ml of alumina (activity grade I deactivated with 6% H_2O): *n*-hexane fraction contained acyclic hydrocarbons; benzene fraction contained aromatic hydrocarbons; $CHCl_3$–MeOH (4:1) fraction contained alcohols; MeOH fraction contained glycerides and other polar lipids.

Urea adduction. Branched hydrocarbons and fatty acids were separated from non-branched components by addition of an aliquot of the hydrocarbon and fatty acid fractions to hot MeOH saturated in urea. After equilibrating for 24 hr the non-branched components were removed with filtration and rinsing with cold benzene and the branched components with extraction with benzene after destruction of the urea clathrates with H_2O.

GLC. Aliphatic, olefinic and branched hydrocarbons and fatty acids as methyl esters were identified and measured by GLC on three 2 m × 0·32 cm (o.d.) copper columns, one packed with 3% SE30, one with 5% DEGS and the other with 5% FFAP all coated on Gas Chrom Q. Identification was effected by comparison of R_ts of both samples and standards on all three GLC phases. Coinjection of authentic standards: *n*-alkanes, *n*-alkenes, pristane, phytane, *iso*-alkanes and fatty acids, further substantiated these identifications.

Hydrogenation. Aliquots of both hydrocarbon and fatty acids fractions were hydrogenated over Adam's Catalyst in hexane. Further confirmation of unsaturated hydrocarbons and fatty acids was achieved by noting the shifts of R_ts to those of the saturated analogues.

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