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ALIPHATIC AND TRITERPENOID HYDROCARBONS FROM FERNS

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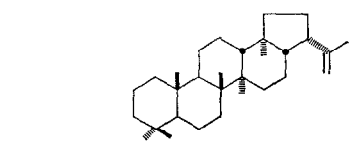
Key Word Index—Filicopsida; chemotaxonomy; *n*-alkanes; hopane derivatives.

Abstract—A detailed analysis of the hydrocarbon fractions from the extracts of 21 ferns belonging to 13 families was carried out. *n*-Alkanes from C_{24} to C_{35} and triterpenoid hydrocarbons of the hopane series were identified. The taxonomic value of these results is discussed.

INTRODUCTION

FERNS (Filicopsida) represent the largest class of Pteridophyta; their classification is rather controversial. Pichi Sermolli has recently proposed[1] to divide this class into 7 subclasses, 24 orders and 55 families. The chemotaxonomy of this class has been investigated only in a rather limited way. Many species contain, beside the usual straight-chain alkanes,[2] several different characteristic triterpenoid hydrocarbons, never found in higher plants: 22(29)-hopene (I),[3] 17(21)-hopene (II),[4] 13(18)-hopene (III),[5] 12-hopene,[5] 11,13(18)-hopadiene,[5] 9(11)-fernene (IV),[6] 8-fernene (V),[3,6] 7-fernene (VI),[3,6] 7,9(11)-fernadiene,[5] adianene,[3,6] 3-filicene (VII),[3,6] and serratene.[7]



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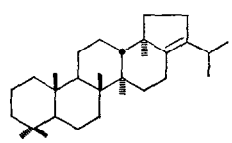

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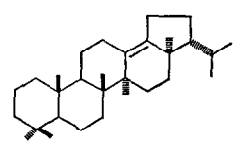

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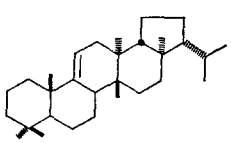

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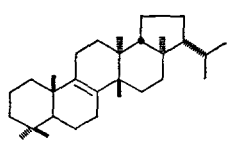

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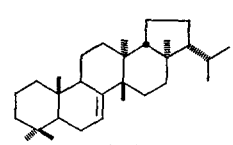

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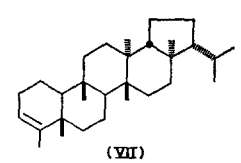

(VII)

Since the isolation of the hydrocarbon fraction from the crude extracts by adsorption chromatography and its analysis by GLC are particularly easy, we have undertaken a study

[1] R. PICHI SERMOLLI, in *Systematics of To-day* (edited by O. HEDBERG), Uppsala Univer., Arsskrift (1958); in *Vistas in Botany* (edited by W. B. TURRILL), London (1959); in *Enciclopedia Agraria Italiana*, Vol. 4, Roma (1960).
[2] G. BERTI and F. BOTTARI, *Prog. Phytochem.* **1**, 589 (1969).
[3] H. AGETA, K. IWATA and S. NATORI, *Tetrahedron Letters* 3413 (1964).
[4] D. H. R. BARTON, G. MELLOWS and D. A. WIDDOWSON, *J. Chem. Soc.* **C**, 110 (1971).
[5] H. AGETA, K. SHIOIMA and Y. ARAI, *Chem. Commun.* 1105 (1968).
[6] H. AGETA and K. IWATA, *Tetrahedron Letters* 6069 (1966).
[7] G. BERTI, F. BOTTARI, A. MARSILI, I. MORELLI and A. MANDELBAUM, *Chem. Commun.* 50 (1967).

of the distribution of aliphatic and triterpenoid hydrocarbons in 21 ferns belonging to 13 families (see Table 1), in a first attempt to establish chemotaxonomic correlations. Figure 1 shows the degree of affinity between these families: the closer the vertical lines, the greater is the affinity of the corresponding families.

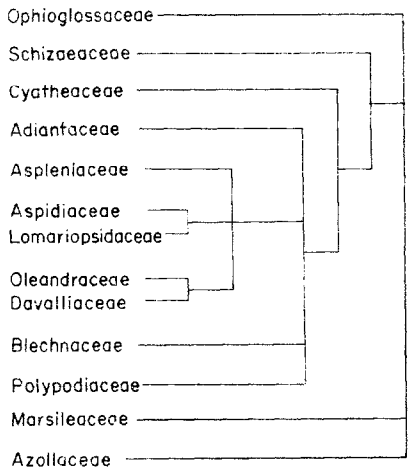

FIG. 1. TAXONOMIC AFFINITIES OF FAMILIES REPRESENTED BY SPECIES EXAMINED.

RESULTS AND DISCUSSION

The results obtained are recorded in Tables 2, 3 and 4. Aliphatic hydrocarbons are widely distributed in the ferns studied, the more abundant being nonacosane (C_{29}) and hentriacontane (C_{31}). The presence of tritriacontane (C_{33}) and pentatriacontane (C_{35}) in some ferns

TABLE 1. SPECIES ANALYSED

Fern	Family	Place of collection	Part used
Oleandra distenta Kunze	Oleandraceae	Ivory Coast	Whole plant
Nephrolepis biserrata (Swartz) Schott	Davalliaceae		
Ctenitis protensa (Afz. ex Swartz) Ching	Aspidiaceae		
Dryopteris filix-mas (L.) Schott		Italy	
Lomariopsis palustris (Hook.) Mett. ex Kuhn	Lomariopsidaceae	Ivory Coast	
Asplenium adiantum-nigrum L.	Aspleniaceae	Italy	
Asplenium africanum Desv.		Ivory Coast	Leaves
Asplenium trichomanes L.			Whole plant
Phyllitis scolopendrium (L.) Newman		Italy	Leaves
Blechnum spicant (L.) Roth	Blechnaceae		Whole plant
Microsorium punctatum (L.) Copel.	Polypodiaceae		Leaves
Platycerium elephantotis Schweinf.			Whole plant
Phymatodes scolopendria (N. L. Burm.) Ching			
Adiantum vogelii Mett. ex Keys.	Adiantaceae		
Cyathea manniana Hook.	Cyatheaceae		Leaves and trunks
Lygodium smithianum Presl ex Kuhn	Schizaeaceae	Ivory Coast	Whole plant
Marsilea diffusa Lepr. ex A. Br.	Marsileaceae		
Marsilea polycarpa Hook. et Grev.			
Marsilea quadrifolia L.			
Azolla africana Desv.	Azollaceae		
Ophioglossum costatum R.Br.	Ophioglossaceae		

is interesting; these compounds were the most important aliphatic constituent of *Phymatodes scolopendria* (Polypodiaceae) and *Lygodium smithianum* (Schizaeaceae), respectively. In many of these ferns several sterols have been found, the main component being sitosterol.

Practically all triterpenoid hydrocarbons found in ferns belong to the hopane (A'-neogammacerane) or rearranged hopane series; the presence of these hydrocarbons appears to be peculiar of primitive plants; such compounds have also been found in mosses[8] and bacteria.[9,10] Two ferns (*Azolla africana* and *Ophioglossum costatum*) contain no hydrocarbons; aliphatic hydrocarbons are absent in *Oleandra distenta* and in *Cyathea manniana*. Two ferns do not contain any triterpenoid hydrocarbons; they are *Asplenium africanum* and *Platycerium elephantotis*. Three ferns (*Nephrolepis biserrata*, *Ctenitis protensa* and *Microsorium punctatum*) contain only 9(11)-fernene and three (*Asplenium trichomanes*, *Lygodium smithianum* and *Marsilea polycarpa*) contain 22(29)-hopene as the only triterpenoid hydrocarbon. All other ferns studied contain from two to five hopane derivatives.

TABLE 2. COMPOSITION OF LIGHT PETROLEUM EXTRACTS

Fern	Waxes (%)	Extract (%)	Unsaponifiable part (%)	Fatty acids (%)	Hydrocarbons (%)	Alcohols +sterols (%)
Oleandra distenta	0·24	1·43	0·65	0·65	0·12	0·48
Nephrolepis biserrata	0·09	0·47	0·35	0·09	0·06	0·26
Ctenitis protensa	0·07	0·85	0·35	0·36	0·07	0·27
Dryopteris filix-mas	0·44	2·34	0·42	1·53	0·08	0·29
Lomariopsis palustris	0·06	0·88	0·40	0·36	0·05	0·31
Asplenium adiantum-nigrum	0·13	1·58	0·61	0·82	0·07	0·44
Asplenium africanum	0·21	2·12	0·66	1·04	0·08	0·51
Asplenium trichomanes	0·36	1·33	0·12	0·95	0·02	0·05
Phyllitis scolopendrium	0·47	2·14	0·17	1·68	0·03	0·12
Blechnum spicant	0·07	0·53	0·16	0·30	0·02	0·13
Microsorium punctatum	0·07	2·65	1·20	0·85	0·09	1·05
Platycerium elephantotis	0·15	2·61	1·05	1·15	0·09	0·71
Phymatodes scolopendria	0·45	3·72	2·35	1·10	0·19	1·79
Adiantum vogelii	0·07	0·67	0·26	0·32	0·04	0·20
Cyathea manniana (leaves)	0·16	1·80	0·80	0·45	0·18	0·50
(trunks)	0·01	0·51	0·18	0·26	0·03	0·10
Lygodium smithianum		1·00	0·40	0·45	0·04	0·30
Marsilea diffusa	0·25	0·70	0·30	0·30	0·04	0·25
Marsilea polycarpa	0·20	0·68	0·40	0·15	0·04	0·30
Marsilea quadrifolia	0·09	0·86	0·27	0·46	0·14	0·09
Azolla africana	0·52	0·32	0·18	0·06		0·17
Ophioglossum costatum	0·06	17·4	0·42	15·0		0·47

From Table 3 it clearly appears that the content of aliphatic hydrocarbons is of no chemotaxonomic value, and the content of triterpenoid hydrocarbons, at least for the ferns studied so far, has only little importance (Table 4). For instance, a high content of 22(29)-hopene (I) has been found in ferns belonging to families which show a certain degree of

[8] (a) A. MARSILI and I. MORELLI, *Phytochem.* **7**, 1705 (1968); (b) *Phytochem.* **9**, 651 (1970); (c) A. MARSILI, I. MORELLI and A. M. IORI, *Phytochem.* **10**, 432 (1971).
[9] M. DE ROSA, A. GAMBACORTA, L. MINALE and J. D. BU'LOCK, *Chem. Commun.* 619 (1971).
[10] C. W. BIRD, J. M. LYNCH, S. J. PIRT and W. W. REID, *Tetrahedron Letters* 3189 (1971).

TABLE 3. COMPOSITION OF THE ALKANE FRACTIONS

Fern	Alkanes (%)	Composition of the alkane fractions									
		C_{24}	C_{25}	C_{26}	C_{27}	C_{28}	C_{29}	C_{30}	C_{31}	C_{32}	C_{33}	C_{35}
Oleandra distenta	0·0											
Nephrolepis biserrata	0·007				27·5	4·5	54·5	1·5	10·5		1·5	
Ctenitis protensa	0·055				3·1	8·9	18·4	3·0	46·0	3·2	17·4	
Dryopteris filix-mas	0·06				4·0	2·0	50·8	3·3	30·3	1·0	8·6	
Lomariopsis palustris	0·02						44·0		44·0		12·0	
Asplenium adiantum-nigrum	0·04				16·0	1·3	24·7	1·5	48·5	0·5	7·5	
Asplenium africanum	0·08				8·6	4·0	25·0	0·6	42·0	0·8	19·0	
Asplenium trichomanes	0·01	3·4	10·6	2·6	11·8	2·7	23·7	2·2	26·6		16·4	
Phyllitis scolopendrium	0·02		1·0		3·5	1·2	45·2	5·7	40·2	0·8	2·4	
Blechnum spicant	0·01		2·4		5·5	1·5	22·3	2·0	46·5		15·0	4·8
Microsorum punctatum	0·06				3·5		18·0	1·5	19·0	2·0	56·0	
Platycerium elephantotis	0·09				3·1		80·7	2·5	13·7			
Phymatodes scolopendria	0·01								9·5		90·5	
Adiantum vogelii	0·03	2·1	2·0	1·4	3·0	1·1	29·3	2·7	31·8	1·7	15·5	9·4
Cyathea manniana	0·0											
Lygodium smithianum	0·005										10·0	90·0
Marsilea diffusa	0·03		7·4	1·5	22·2	2·8	42·8	1·9	15·6		5·8	
Marsilea polycarpa	0·025	4·0	14·0		26·0		44·0		12·0			
Marsilea quadrifolia	0·015		1·5	0·5	39·0	3·5	47·0	1·5	7·0			
Azolla africana	0·0											
Ophioglossum costatum	0·0											

affinity; the same observation may be made for the high content of 9(11)-fernene in other ferns. However, on present data, it appears that the triterpenoid hydrocarbon content can hardly be used as a general chemotaxonomic criterion of classification. It is doubtful that a wider study of the same secondary constituents would alter this conclusion. The fact that plants collected in different places, or in the same place but at different times, may show different ratios of the same triterpene derivatives, as was recently found in the case of a moss,[8c] could also be another factor which renders these substances unsuitable for a general chemotaxonomic classification.

TABLE 4. COMPOSITION OF THE TRITERPENOID FRACTIONS

Fern	Triterpenoid hydrocarbons (%)	Composition of the triterpenoid hydrocarbons fractions						
		(I)	(II)	(III) + (V)	(IV)	(VI)	(VII)	Other components
Oleandra distenta	0·12	36·5			63·5			
Nephrolepis biserrata	0·05				100·0			
Ctenitis protensa	0·01				100·0			
Dryopteris filix-mas	0·01			7·5	81·5	6·5		4·5
Lomariopsis palustris	0·02	17·5		3·0	77·9	1·6		
Asplenium adiantum-nigrum	0·03	90·5		4·1				5·4
Asplenium africanum	0·0							
Asplenium trichomanes	0·01	100·0						8·0
Phyllitis scolopendrium	0·007	88·9					3·1	
Blechnum spicant	0·009	15·5			13·0	5·5		66·0
Microsorum punctatum	0·02				100·0			
Platycerium elephantotis	0·0							
Phymatodes scolopendria	0·175	21·3	10·1	25·3	38·0	5·3		
Adiantum vogelii	0·01	57·5	10·0		7·0	19·5	6·0	
Cyathea manniana	0·21	10·8			53·0	7·2	29·0	
Lygodium smithianum	0·025	100·0						
Marsilea diffusa	0·006	28·5				71·5		
Marsilea polycarpa	0·01	100·0						
Marsilea quadrifolia	0·12	66·0	8·9		25·1			
Azolla africana	0·0							
Ophioglossum costatum	0·0							

EXPERIMENTAL

Quantities of the various plants ranging from 100 g to 5 kg were used. For *Cyathea manniana* the leaves and the trunks were examined separately; for the other ferns either the leaves alone, or the whole plants were extracted (see Table 1). Extractions were made with light petroleum (30–60°) and the extracts were concentrated several times to smaller volumes, mainly to produce separation of waxes. When no more solid

products were obtained, the extracts were evaporated to dryness and the residues saponified. The unsaponifiable fraction, dissolved in light petroleum, was chromatographed on alumina to separate all hydrocarbons from the other more polar constituents (alcohols, sterols). The fatty acids were obtained by acidification of the basic mother liquors from the saponifications. Percentages of the various constituents are shown in Table 2. Separation of aliphatic from triterpenoid hydrocarbons was subsequently carried out by careful chromatography over silica gel impregnated with silver nitrate, using light petroleum and finally benzene as eluants. The various fractions were checked by GLC, using a Carlo Erba Fractovap, mod.G.V. Columns were: for aliphatic hydrocarbons, 1% neopentyl glycol succinate (NPGS) on Chromosorb W 80–100 mesh, programmed temp. 160–220°, increase 5°/min, block temp. 240°, carrier gas N_2, flow rate from 40 to 31 ml/min. For triterpenoid hydrocarbons, 1% NPGS on Chromosorb W 80–100 mesh, column temp. 220°, injection block 260°, carrier gas N_2, flow rate 60 ml/min. and 3% SE-52 on Chromosorb W 80–100 mesh, column temp. 240°, injection block temp. 270°, carrier gas N_2, flow rate 60 ml/min. Retention times relative to cholestane: (II), 2·14; (V), 2·69; (III), 2·78; (IV), 2·98; (VI), 3·95; (I), 5·14; (VII), 5·65 (NPGS); (II), 1·73; (V), 2·00; (III), 2·03; (IV), 2·11; (VI), 2·50; (I), 2·94; (VII), 3·18 (SE-52). When sufficiently pure products were obtained, comparisons with authentic samples were also made both by IR and NMR spectroscopy. More precise details regarding the separation procedures are given in Ref. 8a.

Isolation of n-*pentatriacontane from* Lygodium smithianum. The mixture of aliphatic hydrocarbons obtained from this fern (90% *n*-pentatriacontane and 10% *n*-tritriacontane) was crystallized several times from light petroleum. The C_{35} hydrocarbon had m.p. 71–73°. (Found: C, 85·35; H, 14·63. For $C_{35}H_{72}$ calc.: C, 85·28; H, 14·72.) (Lit.[11] m.p. 74·6°). MS: peaks at *m/e* 492 (M^+), M^+-CH^3 and $M^+-(CH_2)_nCH_3$.

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[11] E. H. Rodd, *Chemistry of Carbon Compounds*, Vol. 1, p. 228, Elsevier, London (1951).