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ISOLATION OF *n*-PARAFFINS AND *sec*-ALCOHOLS FROM *ISOTACHIS JAPONICA**

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Abstract—Two homologous series of *n*-paraffins and *sec*-alcohols from C_{20} to C_{34} or C_{35} were isolated a wax constituents of a liverwort, *Isotachis japonica.* In both series, odd-members were predominant compared with even-members: the ratio of the total amount of odd- to total amounts of even-members was 3·9 in the *n*-paraffin homologues, and 16·5 in the *sec*-alcohol homologues.

INTRODUCTION

It is known that the waxes are widely distributed in both plant and animal kingdoms.[1] However, there are few investigations of the waxes of liverworts (Hepaticae), except in the work reported by Stransky *et al.*[2] and Huneck *et al.*[3] The present paper deals with the isolation of *n*-paraffin and *sec*-alcohol homologues from a liverwort, *Isotachis japonica* Steph. which is a leafy liverwort belonged to the Jungermanniales.

RESULT AND DISCUSSION

A semi-solid substance, which was obtained by hexane-extraction of the liverwort, was chromatographed on a silica gel column with hexane and EtOAc (4:1) as solvent. Three fractions showing homogeneity in TLC analysis were separated in yields of 2·2, 66·7 and 3·3% for the original semi-solid. Of these fractions, the middle main fraction (R_f 0·54) has previously been investigated and characterized as a mixture of aromatic esters.[4] The two remaining fractions were examined in the present work.

n-*Paraffin Homologues*

An oily substance, separated as the first fraction, showed only one spot (R_f 0·75) in TLC. The IR and NMR spectra of this substance could be attributed to the *n*-paraffins:

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* Part X in the series "Chemical Constituents from Hepaticae". For Part IX see A. Matsuo, M. Nakayama, S. Hayashi and S. Yasuda, *Agric. Biol. Chem. Tokyo* **36** (1972). in press.

[1] A. G. Dauglas and G. Eglinton, in *Comparative Phytochemistry* (edited by T. Swain), p. 57, Academic Press, London (1966).

[2] K. Stransky, M. Streible and V. Herout, *Coll Czech. Chem. Commun.* **32,** 3213 (1967).

[3] S. Huneck and E. Klein, *J. Hattori Bot. Lab. Japan* **33,** 1 (1970).

[4] A. Matsuo, M. Nakayama and S. Hayashi, *Z. Naturforsch.* **26b,** 1023 (1971).

ν_{max} 2920, 2860, 1460, 1385 and 720 cm^{-1}; δ_{ppm} 0·90 (*t*) and 1·23 (*s*). In GLC, however, 16 peaks were demonstrated and these components could be assigned to *n*-paraffin homologues from C_{20} to C_{35} by comparison with several authentic *n*-paraffins (C_{18}, C_{22}, C_{24} and C_{28}) and by applying the linear relationship between carbon numbers and retention times. For additional confirmation the substance was subjected to GC–MS and MS were successfully obtained on 10 components (Table 1). All spectra thus obtained were in good agreement with those of the *n*-paraffins in the spectral pattern,[5] and the molecular ions in these spectra confirmed the MW of the components deduced from the R_ts on GLC. Accordingly, it is certain that the fraction eluted most easily in the column chromatography was composed of *n*-paraffin homologues from C_{20} to C_{35}. The relative concentration of these components was calculated from the relative peak areas of the gas chromatogram.

TABLE 1. COMPOSITION OF *n*-PARAFFIN AND *sec*-ALCOHOL HOMOLOGUES IN *Isotachis japonica*

Peak No. of GLC	Carbon atom	*n*-Paraffin	*sec*-Alcohol	Peak No. of GLC	Carbon atom	*n*-Paraffin	*sec*-Alcohol
1	C_{20}	0·4	0·7	9	C_{28}	4·5 (M^+ 394)	1·6
2	C_{21}	0·8	1·5	10	C_{29}	24·4 (M^+ 408)	7·8
3	C_{22}	0·8	0·2	11	C_{30}	4·5 (M^+ 422)	0·9
4	C_{23}	1·6	15·2	12	C_{31}	23·0 (M^+ 436)	15·4
5	C_{24}	2·1	1·6	13	C_{32}	2·9 (M^+ 450)	t
6	C_{25}	5·4 (M^+ 352)	28·2	14	C_{33}	9·3 (M^+ 464)	7·2
7	C_{26}	3·3 (M^+ 366)	1·1	15	C_{34}	1·1	t
8	C_{27}	9·3 (M^+ 380)	18·6	16	C_{35}	3·9	

* The quantitative determination depended on comparison of GLC peak areas.

sec-*Alcohol Homologues*

From the third fraction a solid substance (m.p. 77–78°) was obtained and showed only one spot (R_f 0·25) in TLC. Although the IR and NMR spectra of this substance exhibited the presence of a secondary hydroxyl group: ν_{max} 3300, 1135 and 1095 cm^{-1}; δ_{ppm} 4·02 (*t*, *J* 7·0 Hz), other regions of these spectra resembled closely those of *n*-paraffins. The substance, furthermore, could be converted into an oily acetate (ν_{max} 1735, 1240 and 1035 cm^{-1}) by the treatment with $(Ac)_2O$ in pyridine, and also converted into a ketonic substance (m.p. 70–71°; ν_{max} 1695 cm^{-1}) by oxidation with CrO_3. On the basis of these facts, the substance was identified as a saturated aliphatic *sec*-alcohol. The MS of this substance, however, contained a large number of abundant ions between *m/e* 312 and 494 differing by the same distance of 14 m.u. This suggested that the substance was not a single compound, but was a mixture of saturated *sec*-alcohol homologues having the above abundant ions. The direct gas chromatography of this substance was unsuccessful due to the high b.p. and high polarity. The substance was then subjected to treatment with I_2 and red P followed by reduction with HCl and Zn dust to convert the components into the corresponding saturated hydrocarbons, whose IR and NMR spectra were attributable to *n*-paraffins. The gas chromatographic identification using authentic specimens and the GC–MS combined analysis revealed the reduced hydrocarbons to be a homologous mixture of *n*-paraffins from C_{20} to C_{34}.

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[5] M. J. ONEAL and T. P. WIER, *Analyt. Chem.* **23,** 830 (1951); E. D. MCCARTHY, J. HAN and M. CALVIN, *ibid.* **40,** 1475 (1968).

Therefore, it was concluded that the third fraction separated in the last stage of the elution chromatography was composed of a homologous series of saturated aliphatic *sec*-alcohols from C_{20} to C_{34}, although the position of the hydroxyl group was unknown. Relative concentration of the *sec*-alcohol homologues was calculated from relative peak areas in the gas chromatogram of the reduced hydrocarbon as shown in the fourth column of Table 1.

In the wax of *Isotachis japonica*, the homologues of saturated aliphatic *sec*-alcohols were distributed in nearly the same region from C_{20} to C_{34} as that of the *n*-paraffin homologues (C_{20} to C_{35}), However, the homologous series of alcohol has two distribution maxima at C_{25} and C_{31}, while the *n*-paraffin series has one maximum at C_{29}. In both series of *n*-paraffins and *sec*-alcohols, the odd-members were commonly predominant compared with both adjacent even-members,[6] and this tendency was especially remarkable in the alcohol homologues: the ratio of the total amount of odd- to the total amount of the even-member series was 3·9 in the *n*-paraffin homologues, whereas it was 16·5 in the *sec*-alcohol homologues. The lower value of this ratio in the *n*-paraffin homologues indicates that the liverworts are low in the evolutionary scale.[2]

EXPERIMENTAL

The IR were taken in CCl_4 or KBr pellets, and the NMR on a 60 MHz spectrometer in $CDCl_3$ by using TMS as an internal standard. MS were recorded on a Hitachi single focus apparatus operated at 80 eV ionization voltage and 150° ionization chamber temperature using a direct inlet system.

Material. The liverwort, *Isotachis japonica*, was collected at Yakushima in S. W. Japan, and dried in the shade for several days. The dried plants (1500 g) were digested with hexane (15 l.) for 1 week at room temp. to obtain a dark green semi-solid (2·7 g) which showed 3 spots (R_f 0·25, 0·54 and 0·75) in TLC on silica gel with a solvent of hexane and EtOAc (4:1).

Separation of n-*paraffin and* sec-*alcohol fractions.* The above semi-solid substance (1·0 g) was applied on a column (1·9 × 50 cm) of silica gel (50 g) and eluted with a mixture of hexane and EtOAc (4:1) to be separated into 3 fractions. A fraction (22 mg) emerging first from the column showed IR and NMR characteristic of *n*-paraffins: ν_{max} 2920, 2860, 1430, 1380 and 720 cm^{-1}; δ_{ppm} 0·90 (*t*, *J* 5·0 Hz) and 1·23 (*s*). The middle fraction (667 mg) was obtained as a viscous oil.[3] The last fraction (33 mg) was obtained as a solid substance (m.p. 77–78°), and showed IR and NMR attributable to *sec*-alcohols: ν_{max} 3300, 2920, 2860, 1465, 1380, 1135, 1095 and 720 cm^{-1}; δ_{ppm} 0·90 (*t*, *J* 5·0 Hz), 1·23 (*s*), 2·22 (complex) and 4·02 (*t*, *J* 7·0 Hz). MS of the last fraction contained a large number of abundant ions: *m/e* 494 ($C_{34}H_{70}O$), 480 (C_{33}), 466 (C_{32}), 452 (C_{31}), 438 (C_{30}), 424 (C_{29}), 410 (C_{28}), 396 (C_{27}), 382 (C_{26}), 368 (C_{25}), 354 (C_{24}), 340 (C_{23}), 326 (C_{22}) and 312 (C_{21}).

GLC analyses of n-*paraffin fraction.* The paraffin fraction was reported on a stainless steel column (2 mm × 2 m) packed with SE30 (10%) on Diasolid L (60–80 mesh) or silicon OV-1 (1%) on Uniport B (60–80 mesh), with temp. programming at 3°/min from 150° to 300° at 15 ml/min flow rate of N_2. The fraction showed 16 peaks by FID.

GC-MS analyses of the n-*paraffins and the reduced product of the sec-alcohol fraction.* The GC-MS analyses were performed with a single focus MS under the following conditions: silicon SE30 on Diasolid L (2%) or silicon OV-1 on Uniport B (1%); column temp. programming at 2°/min from 150 to 300°; 0·8 kg/cm^2 pressure of He carrier; 80 μA total emission; 1500 eV ion accelerating volatge and 200° ionization chamber temp.

Acetylation of the sec-*alcohol fraction.* The alcohol fraction (10 mg) was mixed with Ac_2O (0·1 ml) and pyridine (0·3 ml), and the mixture allowed to stand overnight at room temp. The reaction mixture, after being diluted with H_2O (3 ml), was extracted with light petrol. (5 ml × 2) to obtain an acetylated product as a semi-solid substance: ν_{max} 1735, 1465, 1240, 1035 and 725 cm.$^{-1}$

Oxidation of the sec-*alcohol fraction.* The *sec*-alcohol fraction (30 mg) was dissolved in AcOH (2 ml) and mixed a solution of CrO_3 (10 mg) in AcOH (1 ml). After standing at 60° for 10 min, H_2O (10 ml) was added, and the mixture extracted with light petrol. (3 ml × 5). The combined petrol solution was evaporated to obtain a ketonic substance: m.p. 70–71°; ν_{max} 1695, 1465, 1380 and 725 cm^{-1}.

Reduction of the sec-*alcohol fraction into the* n-*paraffin mixture.* The *sec*-alcohol fraction (40 mg) was heated together with I_2 (30 mg) and fed P (10 g) in a sealed tube at 100° for 3 hr. The reaction mixture

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

was extracted with light petrol. The solution, after being filtered through a small column packed with silica gel, was refluxed with 0·5 N methanolic HCl (5 ml) and Zn dust (0·5 g) for 2 hr, occasionally adding further small amount of the reducing agents. The reaction mixture was extracted into light petrol. The solution was filtered through a small column of silica gel, and a reduced product was obtained as an oily substance. The IR and NMR spectra were attributable to *n*-paraffins: ν_{max} 2920, 2860, 1460, 1380 and 720 cm^{-1}; δ_{ppm} 0·90 (*t*, *J* 5·0 Hz) and 1·23 (*s*), but GLC showed 15 peaks.