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GAS–LIQUID CHROMATOGRAPHY OF TERPENES—XIV.

THE CHEMICAL COMPOSITION OF THE VOLATILE OIL OF THE LEAVES OF *PICEA RUBENS* SARG. AND CHEMOTAXONOMIC CORRELATIONS WITH OTHER NORTH AMERICAN SPRUCE SPECIES*

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Abstract—The volatile oil from two populations of red spruce was found to consist mainly of bornyl acetate (55 per cent). Smaller amounts of α- and β-pinene, camphene, 3-carene, myrcene, limonene, α- and β-phellandrene, *p*-cymene, 1:8-cineole, linalool, camphor, terpinen-4-ol, α-terpineol, borneol, piperitone, citronellyl and geranyl acetate, bisabolene and a mixture of γ- and δ-cadinene ("canadene") were also isolated. Santene, tricyclene, γ-terpinene, thujone, isothujone and isoborneol were tentatively identified. The leaf oils of the foliage from black spruce (2 trees) white spruce (3 trees) and Colorado spruce (5 trees) were analysed by GLC and the data obtained were compared with those of red spruce. The close phylogenetic relationship of red and black spruce was found to be reflected in the chemical composition of their leaf oils, whereas significant chemical differences were observed with both white and Colorado spruce. Hypothetical pathways for the formation of the terpenes found in spruce leaf oils from a single precursor, such as geranyl pyrophosphate, are discussed. Piperitone, a C-3 oxygenated monoterpene, appears to take an exceptional position in such postulated sequences. Also, not all of the compounds expected from schemes involving carbonium-ion intermediates are found in the spruce leaf oils.

INTRODUCTION

RED SPRUCE (*Picea rubens* Sarg.) occurs in the north-eastern regions of the North American continent and overlaps the ranges of black (*P. mariana* (Mill) B.S.P.) and white (*P. glauca* (Moench) Voss) spruce,[1] and natural hybrids between red and black spruce are known to occur.[2] Wright[1] refers to a personal communication according to which hybrids between red and white spruce have also been found. Wright considers red and black spruce to be probably remnants of a more ancient migration than that which gave rise to the spruce species of north-western America (white, Colorado, Engelmann and Sitka spruce).

Previously the author has analysed the volatile oils of the leaves of black, white and Colorado (*P. pungens* Engelm.) spruce,[3] as well as those of Sitka (*P. sitchensis* (Bong.) Carr.) and Engelmann (*P. engelmannii* Parry) spruce[4] and it was shown[4,5] that the chemical components of conifer leaf oils may be used for chemo-taxonomic correlations. It was of interest to determine if the above botanical relationship based on morphology could be correlated with the chemical composition of the oils of these spruce species. As the literature is devoid of any reference to the chemical composition of red spruce leaves a detailed analysis of the volatile oil from red spruce was carried out. The foliage from two populations (A and B)

* Issued as N.R.C. No. 8890.

[1] J. W. WRIGHT, *Forest Sci.* **1**, 319 (1955).

[2] C. C. HEIMBURGER, *Forest Chron.* **15**, 226 (1939); see also E. K. MORGENSTERN and J. L. FARRAR, *Forest Res. Branch Contrib. No.* 608, Canadian Dept. of Forestry (1964).

[3] E. VON RUDLOFF, *Tappi* **45**, 181 (1962).

[4] E. VON RUDLOFF, *Can. J. Chem.* **42**, 1057 (1964).

[5] E. VON RUDLOFF, *Can. J. Chem.* **41**, 1737, 2876 (1963).

located in the Monongahela National Forest, West Virginia, and the North Carolina side of the Great Smoky Mountain National Park was investigated.

From previous work[3] it appeared that the seasonal variation in the composition of spruce leaf oils was least during the fall and winter months, although a gradual decrease in the yield of oil must be expected as winter progresses. Collection of foliage during the colder part of the year also reduces any possible loss or deterioration during transport. Furthermore, metabolic processes being then at a minimum, a comparison of chemical components from different species may have more phytochemical value, and better reproducibility within the same species is to be expected.

In addition to the red spruce leaf oil, small amounts of the oil from local white, black and Colorado spruce were analysed by gas–liquid chromatography (GLC) as described previously[4, 6] to facilitate direct comparison. To obtain some idea as to the variation from one tree to another the leaf oils from several individual trees were investigated.

The foliage from the two red spruce populations was divided into a number of batches and analysed by the same technique. The oil was then divided into hydrocarbons and oxygenated terpenes by chromatography on deactivated silicic acid,[6, 7] and individual components were isolated by preparative GLC. Known terpenes were identified by comparison of physical constants, including relative retention times (r.r.t.) on different GLC columns, and i.r. spectra.

RESULTS

A typical chromatogram (polyethylene glycol PEG 20M temperature-programmed from 55 to 200° at 4°/min) of the red spruce leaf oil is shown in Fig. 1. The overlap of hydrocarbons and oxygenated terpenes is indicated by the peaks shown in solid black, viz. 1:8-cineole (peak 10) in the monoterpene hydrocarbon range and three sesquiterpenes (peaks 30, 33a and 34) in the range of the oxygenated monoterpenes. The average percent composition of the oils from the two different red spruce populations A and B is shown in Table 1. Components which were identified only tentatively are shown in parentheses.

The results obtained show that the main constituent is bornyl acetate (V). Smaller amounts of camphene (VI), α-pinene (XVI), 3-carene (XIV), myrcene, limonene (XII), β-phellandrene, *p*-cymene, β-pinene (XVII), α-phellandrene, borneol, piperitone, terpinen-

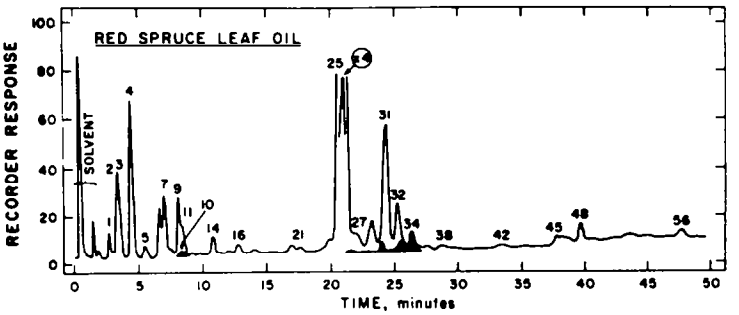


FIG. 1. GAS CHROMATOGRAM OF THE VOLATILE OIL FROM THE LEAVES OF RED SPRUCE (180 × 0·3 cm POLYETHYLENE GLYCOL 20m COLUMN, TEMPERATURE-PROGRAMMED FROM 55° TO 200° AT 4°/min).

[6] F. M. COUCHMAN and E. VON RUDLOFF, *Can. J. Chem.* **43**, 1017 (1965).
[7] E. KUGLER and E. S. KOVATS, *Helv. Chim. Acta* **46**, 1480 (1963).

TABLE 1. PERCENTAGE COMPOSITION OF THE VOLATILE LEAF OIL OF RED SPRUCE FROM TWO GEOGRAPHIC REGIONS

Peak No.	Compound	A* (%)	B† (%)	Peak No.	Compound	A* (%)	B† (%)
1	(Santene)	1·0	1·3	30a	C_{15} Hydrocarbon	0·3	0·2
2	(Tricyclene)	0·8	1·0	30b	α-Terpineol	2·5	2·5
3	α-Pinene	3·4	2·5	31	Borneol	5·5	4·8
4	Camphene	6·3	6·8	32	Piperitone	2·5	3·6
5	β-Pinene	0·5	0·4	33a	Bisabolene	0·3	0·2
6	3-Carene	2·0	1·0	33b	Citronellyl acetate	0·4	0·2
7	Myrcene	2·2	2·3				
8	α-Phellandrene	0·2	0.1	34	γ+δ-Cadinene	1·2	1·0
9	Limonene	2·2	2·5	35	Geranyl acetate	0·3	0·1
10	1:8-Cineole	0·2	0·4	36		0·2	0·3
11	β-Phellandrene	1·0	1·2	37		0·1	0·3
12	(γ-Terpinene)	0·2	trace	38	>C=O	0·2	0·3
13	(Terpinolene?)	0·1	trace	39		?	?
14	p-Cymene	0·8	0·9	40	(Artefact?)	trace	trace
15		0·2	0·2	41		0·1	0·1
16		0·5	0·4	42		0·2	?
17	(Thujone)	0·2	0·2	43		trace	trace
18	(Isothujone)	0·1	0·1	44		trace	trace
19		0·5	trace	45	Aliphatic acetates	0·5	0·6
20		0·5	0·3	46		0·4	0·6
21	Camphor	0·3	0·2	47	(Cardinol?)	0·4	0·4
22		trace	0·1	48	−OH, −COOAc	1·2	0·8
23		0·1	0·2	49		0·2	0·2
24	Linalool	0·9	1·1	50		trace	trace
25a	Alcohol I	0·5	0·5	51		trace	trace
25b	Bornyl acetate	54·0	56·0	52		0·3	0·2
26	Terpinene-4-ol	1·0	1·0	53	(Artefact?)	trace	trace
27		1·0	0·4	54		—	—
28	(Isoborneol)	0·6	0·9	55		—	—
29	Alcohol II	1·6	1·2	56	OH, >C=O	0·6	0·6

* From Monongahela National Forest, West Virginia.
† From Great Smoky Mountain National Park, North Carolina.

4-ol (XI), α-terpineol (XIII), linalool, camphor, citronellyl acetate, geranyl acetate, 1:8-cineole, bisabolene and a mixture of γ- and δ-cadinene were also identified (Tables 2 and 3). This mixture of γ- and δ-cadinene appears to be the "canadene" isolated by Shaw from black spruce[8] and hemlock[9] leaf oils. These two cadinenes can be resolved partially on Apiezon N(AN) or polyester (NGA, SEG) columns and the i.r. spectrum of the mixture shows clearly all the absorption bands of the two pure compounds. Herout and Sykora[10] report optical rotations of +94° and +148° for γ- and δ-cadinene respectively and the samples used for comparison in this study had values of +80° and +150°. However, the mixture isolated from the red spruce oil had a rotation of only +43° and this suggests that one of the cadinenes in the mixture is an optical enantiomer. In the initial analyses, alcohol I (peak 25a; Table 1, Fig. 1) and terpinene-4-ol (peak 26) were not resolved from bornyl acetate, and α-terpineol (peak 30b) was not resolved from borneol (peak 31). However, analysis of the oxygenated terpene fraction on a 300 × 0·3 cm PEG column did resolve these three com-

[8] A. C. SHAW, *Can. J. Res.*, **B28**, 268 (1950).
[9] A. C. SHAW, *J. Am. Chem. Soc.* **73**, 2859 (1951).
[10] V. HEROUT and V. SYKORA, *Tetrahedron* **4**, 246 (1958).

TABLE 2. PHYSICAL CONSTANTS OF THE HYDROCARBONS OF RED SPRUCE LEAF OIL

Peak No.	Compound	$[\alpha]_D$	n_D	Isooct. λ_{max}	Relative retention times* column AN†	PEG‡	EGPN§	RO‖
1	(Santene)	—	—	—	0·32	0·25	0·22	—
2	(Tricyclene)	−7·9°	1·4658	—	0·39	0·29	0·24	0·41
3	α-Pinene			—	0·42	0·29	0·30	0·45
4	Camphene	−41·8°	1·4948	—	0·50	0·41	0·44	0·55
5	β-Pinene	+32	—	—	0·63	0·55	0·54	0·64
6	3-Carene	−5·2°	1·4701	—	0·82	0·73	0·67	0·81
7	Myrcene			225	0·60	0·82	0·88	0·69
8	α-Phellandrene			267	0·82	0·82	0·86	0·86
9	Limonene	−28·7	1·4758	232	1·00	1·00	1·00	1·00
11	β-Phellandrene				0·97	1·06	1·16	1·05
12	(γ-Terpinene)	—	—	—	1·11	1·40	1·41	1·25
13	(Terpinolene)	—	—	—	1·30	1·63	1·75	1·46
14	p-Cymene	—	1·4925	272	1·0	1·75	2·32	1·07
30a	Unidentified	+9·2°	1·4896	—	1·04	1·06		
33a	Bisabolene	−17·8°	1·4991	—	1·25	1·66		
34	γ- and δ-Cadinene	+42·7°	1·5008	—	1·36 / 1·38	1·90	¶	

* Monoterpenes (peaks 1–14) measured relative to limonene; sesquiterpenes (peaks 30a, 33a and 34) relative to cedrene.
† 10% Apiezon N column (180 × 0·4) at 65° or 150°.
‡ 15% Polyethylene glycol column at 65° or 180°.
§ 15% Ethylene glycol *bis*-propionitrile column at 65°.
‖ 10% Rapeseed oil column (300 × 0·4) at 100°.
¶ r.r.t. on a 1% ethylene glycol succinate polyester (PVP modified) column (300 × 0·3 cm) = 2·56 and 2·46; on 15% neopentyl glycol adipate polyester column (180 × 0·4 cm) = 1·92 and 1·85.

TABLE 3. PHYSICAL CONSTANTS OF THE OXYGENATED MONOTERPENES OF RED SPRUCE LEAF OIL

Peak No.	Compound	$[\alpha]_D$	n_D	Relative retention times column AN*	PEG†	NGA‡	QF-1§
10	1:8-Cineole	—	—	0·55	0·21	0·25	0·12
21	Camphor	—	—	1·00	1·00	1·00	1·00
24	Linalool	+6·3°	1·4623	0·69	1·02	0·92	0·24
25a	Alcohol I	+2·5°	1·4810	1·16	1·32	1·31	0·30
25b	Bornyl acetate	−21·1°	1·4613	2·06	1·35	1·45	1·38
26	Terpinen-4-ol	±0°	1·4782	1·40	1·42	1·39	0·40
28	(Isoborneol)	—	—	1·23	1·94	1·59	0·44
29	Alcohol II	+2·05	1·4908	1·35	2·04	1·60	0·49
30b	α-Terpineol‖	−47·4°	1·4809	1·50	2·20	2·05	0·49
31	Borneol¶	−18·4°	—	1·28	2·28	1·84	0·51
32	Piperitone	−16·8°	1·4793	2·35	2·57	2·31	2·15
33b	Citronellyl acetate	—	—	3·53	2·08	—	1·58
35	Geranyl acetate	—	—	4·5	3·38	—	1·90

* Apiezon N column (180 × 0·4 cm) at 110°.
† 15% Polyethylene glycol column at 120°.
‡ 15% Neopentyl glycol adipate polyester column at 120°.
§ 15% Fluorinated silicone polymer column at 100°.
‖ Melting point 27–29°.
¶ Melting point 175–182°.

ponents partially. By using the QF-1 (fluorinated silicone polymer) and NGA (neopentyl glycol adipate polyester) columns almost pure terpinen-4-ol and α-terpineol were obtained, but the two unidentified alcohols I and II (25a and 29, Table 1) were not sufficiently pure for positive identification. The i.r. spectrum of the impure alcohol I suggests it could be a cyclic alcohol with a trisubstituted double bond (1642, 815 cm^{-1}) and the broad singlet at 1377 cm^{-1} shows it cannot have an isopropyl or *gem*-dimethyl group. Alcohol II was not obtained free from peak 28 and its i.r. spectrum suggests that the latter could be isoborneol. This is in agreement with the r.r.t. values. Alcohol II had no characteristic absorption for a di- or trisubstituted double bond and the singlet at 1380 cm^{-1} indicated absence of an isopropyl

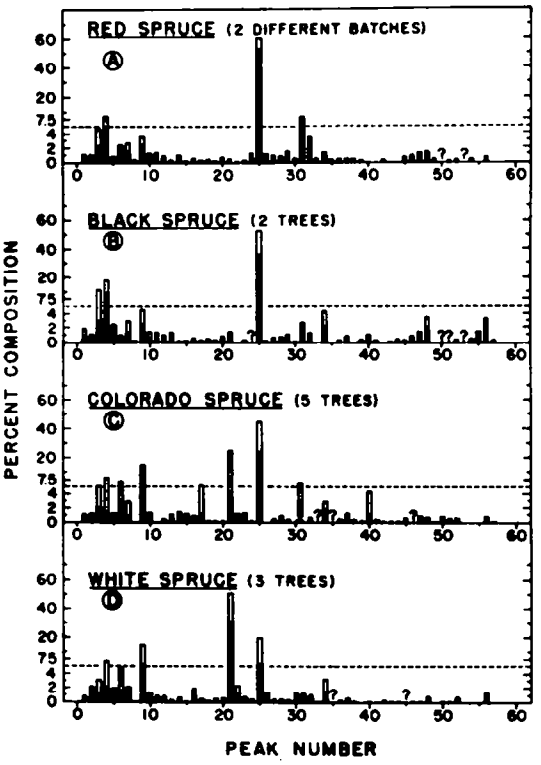


FIG. 2. MAXIMUM AND MINIMUM PERCENTAGE COMPOSITION OF THE VOLATILE OILS FROM RED (A), BLACK (B), COLORADO (C), AND WHITE (D) SPRUCE FOLIAGE. THE SCALE BELOW 10 PER CENT IS ENLARGED.

or *gem*-dimethyl group. The i.r. spectra and r.r.t. data show that alcohols I and II must be different from the terpineols, terpinenols, isopulegol, dihydrocarveol, carveol, sabinol, pulegol, or piperitol.

Components in the oxygenated sesquiterpene range (peaks 40 to 56) could not be obtained in amounts sufficient for adequate purification. Components 45, 46 and 48 appear to be acetates, whereas peak 47 may represent a cadinol. The i.r. spectrum of peak 56 showed the presence of both hydroxyl and carbonyl (1705 cm^{-1}) functions.

The maximum and minimum amounts of each peak recorded for the leaf oil from the various batches of the two red spruce populations A and B are shown diagrammatically in Fig. 2A. This schematic arrangement (peaks in the same order as recorded on the PEG

column, Fig. 1) was found to facilitate direct comparison of the composition of the leaf oil of red spruce with that of the oils from the other spruce species investigated. The scale below 10 per cent is enlarged to show the minor and trace components more clearly. The results obtained by GLC analysis (maximum and minimum values shown as above) for the oils from two black spruce trees, five Colorado spruce trees, and three white spruce trees are shown in Fig. 2B, C and D, respectively. The data obtained are in good agreement with those obtained previously,[4] except for the minor differences found for the Colorado spruce oils as outlined below. It is noteworthy that the yield of leaf oil obtained from each species varied considerably from batch to batch (e.g. red spruce 0·34 to 0·88%, Colorado spruce 0·04 to 0·09%), whereas the quantitative variation of the individual components in each oil was not large, except perhaps for those of the five Colorado spruce trees. The larger variation of the latter may be due to these trees originating from nursery seedlings. The quantitative variation found in the different batches of red spruce leaf oil exceeded only slightly the experimental error.[11] All major and minor components of a particular species were recorded in each batch except for two or three trace components (peaks 36, 38 and 47) in the Colorado spruce oils which appeared to be absent in two of the five samples and an additional minor component in the camphor peak which was not found before. Also, in each sample of the Colorado spruce leaf oil peaks 30 and 31 were poorly resolved and it is possible that a third component was present.

DISCUSSION

The composition of the leaf oil of red spruce from the two different locations did not differ to any significant extent (see Table 1) and resembles closely that of the oil from black spruce foliage[4] (compare Fig. 2A and B). Allowing for the quantitative variations from tree to tree within the same species, the difference between these two spruce leaf oils appears negligibly small. However, since it was found that each batch of red spruce foliage (from both population A and B) contained the same major, minor and trace components it could well be that it is the minor qualitative differences found between the red spruce and black spruce oils that reflect phylogenetic differences. Thus, the minor components (numbered as in Table 1) 22, 24 (linalool), 25a (alcohol I), 33, 49 and 51 were found only in red spruce oil, whereas the minor components 40, 41, 43, 44 and 55 found in black spruce oil were absent. The amounts of these components are far too small to permit positive identification and it follows that differentiation of these two oils will be difficult and detection of hybridization by means of the chemical composition of the leaf oil[4] may require a much more refined technique for these two species.

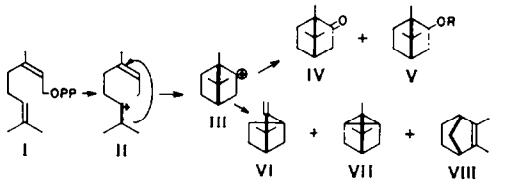
The chemical differences found between red spruce leaf oil and those of Colorado (cf. Fig. 2C) and white spruce (cf. Fig. 2D) are much more distinct, since the latter two contain major amounts of camphor and fewer components (as well as lesser amounts) in the oxygenated sesquiterpene (peak 40 to 58) range. The presence of larger amounts of limonene may also be significant. Comparison of the composition of red spruce leaf oil with that of Engelmann and Sitka spruce[4] shows even greater differences. Hence, the close phylogenetic relationship between the two north-eastern species, *P. rubens* and *P. marina*, as well as their phylogenetic differences with the north-western species, *P. glauca*, *P. pungens*, *P. engelmannii* and *P. sitchensis* (as discussed by Wright[1]) are clearly reflected in the chemical composition of the

[11] J. JANAK, *J. Chromatog.* 3, 308 (1960).

leaf oils. Many more individual trees from different populations will have to be investigated to determine whether this conclusion holds generally. However, even the limited data obtained in this study show a remarkably constant qualitative and reasonably consistent quantitative composition of the leaf oils of each species, and the results obtained further strengthen the previous conclusion that the conifer leaf oils may have considerable chemotaxonomic value. Also, whereas detection of hybridization by means of the leaf oil composition between red and black spruce appears to be very difficult, hybrids between red and white spruce[1] might be detected fairly readily. Detection of hybridization between white and Engelmann spruce by this technique is currently being studied and preliminary results confirm the feasibility of this approach.

Ruzicka[12] has proposed chemical pathways by which the monoterpenes may be formed from a common precursor of the geraniol type and has recently enlarged upon this hypothesis by formulating the biogenetic isoprene rule.[13] According to this, 2- and 3-isopentenyl pyrophosphate combine to give geranyl pyrophosphate which is postulated to be the precursor of most of the common monoterpenes. This view has received considerable experimental support in recent years,[13, 14] although the results obtained by Sandermann *et al.*[15, 16] indicate that the cyclization step may involve a different mechanism than originally proposed by Ruzicka. For the terpenes found in the genus *Mentha*, Reitsema[17] proposed a pathway in which an aliphatic precursor is converted to a cyclic one which gives rise to the sequence: →piperitenone→piperitone→pulegone→menthone→menthol→menthyl acetate (in *M. piperita* L.). Bataille and Loomis[18] confirmed that such a sequence is operative. The acyclic precursor may also give rise to the acyclic monoterpenes found in mint oils.[17]

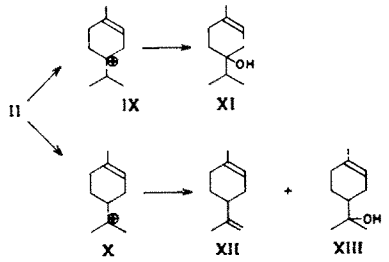
Previously,[4] it was noted that the leaf oils of members of the family *Pinaceae* are characterized by the presence of camphor, borneol, and bornyl acetate. To these one may add as typical minor components camphene, tricyclene and santene. These terpenes not only are constituents of the leaf oils of the genus *Picea*, but have also been detected by Shaw in leaves of *Tsuga canadensis* (L.) Carr[9] and *Abies balsamea* (L.) Mill.[19] If the monoterpenes found in these conifer oils are formed from a single precursor such as geranyl pyrophosphate then one may consider as a basic pathway the sequence geranyl pyrophosphate (I)→intermediates of type II and III→camphor (IV), borneol and bornyl acetate (V), or→camphene (VI), tricyclene (VII), and santene (VIII) (Scheme 1).



SCHEME 1

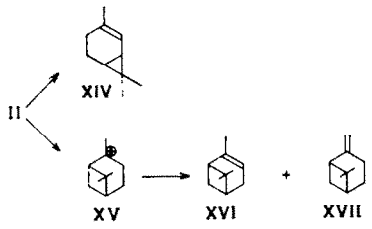
[12] L. RUZICKA, *Experientia* **9**, 357 (1953).
[13] L. RUZICKA, *Proc. Chem. Soc.* 341 (1959); *Pure Appl. Chem.* **6**, 493 (1963).
[14] J. H. RICHARDS and J. B. HENDRICKSON in *The Biosynthesis of Steroids, Terpenes, and Acetogenins*. Benjamin, New York (1964).
[15] W. SANDERMANN and W. SCHWEERS, *Tetrahedron Letters* No. 7, 257 (1962).
[16] W. SANDERMANN and K. BRUNS, *Tetrahedron Letters* No. 7, 259 (1962).
[17] R. H. REITSEMA, *J. Pharm. Sci.* **47**, 267 (1958); **50**, 18 (1961).
[18] J. BATAILLE and W. D. LOOMIS, *Biochim. Biophys. Acta* **51**, 545 (1961).
[19] A. C. SHAW, *Can. J. Chem.* **31**, 193 (1953).

The non-classical carbonium-ion II may also cyclize to the ions IX and X, which are the postulated[12] precursors of terpinen-4-ol (XI), limonene (XII), and α-terpineol (XIII) (Scheme 2). The two intermediates IX and X are also the postulated precursors of the terpinenes and



SCHEME 2

terpinolene, which were not found in any significant amount in the spruce leaf oils. From a purely chemical point of view, larger amounts of the terpinenes and terpinolene would be expected if IX (and X) are intermediates. The difference between expectation based on chemical data and the actual situation in the plant may be accounted for by specific enzyme action, or it may also indicate that carbonium-ions play little or no role in terpene biosynthesis. The presence of 3-carene and the pinenes may result from carbonium-ion II cyclizing directly to give 3-carene (XIV) or from ion XV, which is the chemical precursor[12] of the pinenes (XVI and XVII) (Scheme 3).



SCHEME 3

In addition one must consider for the spruce leaf oils a biosynthetic sequence leading to the acyclic terpenes myrcene, linalool, citronellol, geraniol and the acetates of the latter two, which could result from direct hydrolysis of geranyl pyrophosphate. In Sitka spruce leaf oil myrcene is one of the major constituents,[4] showing that this sequence may become predominant. However, it is not known whether the formation of myrcene proceeds via the oxygenated terpenes, e.g. by dehydration of linalool, or by an independent pathway.

Previously it was thought that piperitone, the third major constituent of the oil from Sitka spruce,[4] is an atypical component of the spruces. However, significant amounts (2–4 per cent) of piperitone have now been isolated from red spruce leaf oil and comparative GLC analysis indicates that this ketone (peak 32, Fig. 2) is also present in black, white and Colorado spruce foliage (up to 1 per cent). Hence, oxidation of a *p*-menthene precursor at C-3 appears to be an integral part of the biosynthesis of monoterpenes in the genus *Picea*. A possible mode of formation of piperitone may be cyclization of geranyl pyrophosphate without loss of the oxygen function, i.e. without carbonium-ion intermediates of type II or III. A study of the formation of piperitone in plants may thus be of particular interest. Also

of interest would be to determine whether the hydrocarbons are formed from the same intermediates as those leading to the oxygenated monoterpenes. Sitka spruce leaf oil containing about 20 per cent each of myrcene, camphor and piperitone may offer a good basis for such study, whereas the oil of red or black spruce leaves may be suitable for a study of the pathway through to borneol and its acetate. However, a disadvantage in using these species may be the relatively low yields of most conifer oils and, perhaps, a slower rate of production as compared with, for example, the mint plants.[17,18]

EXPERIMENTAL

Linear temperature-programmed GLC analyses (1–3 μl aliquots) were carried out with an F & M model 500 (F & M Scientific Corp.) instrument, using 180 (or 300) × 0·3 cm i.d. coiled copper or stainless-steel columns and helium as carrier gas. Oil samples and fractions thereof were analysed on PEG (15% Carbowax 20M on Gaschrom P, 60–80 mesh) and AN (10% Apiezon N) columns from 55° to 200° at 4°/min. Isothermal runs (hydrocarbons at 55–65°, oxygenated monoterpenes at 100–120°, sesquiterpenes at 150–180°) were carried out with a conventional instrument using 180 or 300 × 0·4 cm columns with EGPN (15% ethylene glycol *bis*-propionitrile), NGA (15% neopentyl glycol adipate polyester), QF-1 (15% fluorinated silicone polymer) or RO (10% rapeseed oil) as liquid phase. The per cent composition of each oil was determined by measurement of the area under the peaks (triangulation method) as recorded in duplicate runs on the PEG column. The ratio of peaks, which were not resolved on this column, was obtained by similar measurement of the resolved peaks from chromatograms obtained on other columns. Relative retention times of monoterpene hydrocarbons were measured with respect to limonene, of oxygenated monoterpenes relative to camphor, and sesquiterpenes relative to cedrene or cedrol. Preparative GLC runs were carried out with an Aerograph model A-700 Autoprep unit (Wilkins Instrument and Research Inc.) using 300 or 600 × 0·9 cm o.d. coiled aluminum columns containing 20–25 per cent SE-30 (silicone rubber), PEG, or QF-1 on Chromosorb W (60–80 mesh). Fractions which were not resolved on these columns were further fractionated on the 0·4 cm i.d. analytical columns using hand collection.

Infra-red spectra were recorded (films between sodium chloride plates) with a Perkin–Elmer model 21 double beam spectrophotometer. Optical rotations were determined either with undiluted samples (neat) or in chloroform solution (3–5 per cent) at 24–26°.

Plant Material

The first shipment of red spruce foliage (25·2 kg) was obtained from twelve mature trees located in the Monongahela National Forest, West Virginia, U.S.A., during February, 1964. A second shipment was collected near Newfound Cap on the North Carolina side of the Great Smoky Mountain National Park at the end of February, 1964. The latter foliage (12·6 kg) came from twelve young and mature trees growing at an altitude of 1500 m.

Foliage from Colorado spruce (five mature trees) and white spruce (one mature tree) came from the grounds of the University of Saskatchewan, Saskatoon. That of black spruce (two mature trees) and white spruce (two mature trees) was collected from natural populations at Loon Lake and Candle Lake, Saskatchewan (one tree each from each location).

Isolation of the Leaf Oils

The twigs and leaves were detached from any woody branches and were steam-distilled exhaustively (5–6 hr) in 2 kg batches in a stainless-steel apparatus. The distillate was satu-

rated with sodium chloride and extracted with three portions of ether. The ethereal extract was washed with a little sodium bicarbonate solution (little or no reaction was noticed) and water, and was then dried over anhydrous sodium sulphate. The ether was removed by distillation through a double-walled air-condenser, maintaining the pot temperature below 60° to retain any low boiling leaf oil constituents. The leaf oil from the red spruce population A (10 batches) was obtained in 0·34 to 0·69 per cent yield (n_D 1·4677 to 1·4695, α_D −8·4 to −13·8) and that of population B (5 batches) in 0·61 to 0·88 per cent yield (n_D 1·4665 to 1·4675, α_D −5·0 to −7·7°) based on the fresh weight of foliage. Each batch was analysed by GLC and the maximum and minimum values for each peak recorded (PEG column) are shown digrammatically in Fig. 2A. The individual batches from population A and B were combined (samples I and II, respectively) and the average per cent composition for each population is shown in Table 1.

The foliage (~500 g each tree) from mature black (2), Colorado (5), and white (3) spruce trees was collected during October–December, 1964, and steam distillation gave a yield of 1·25 to 1·85 per cent (n_D 1·4735 to 1·4738; α_D −27·0 to −29·0°), 0·04 to 0·09 per cent (n_D 1·4705 to 1·4777, α_D −10·3 to −29·9°) and 0·10 to 0·18 per cent (n_D 1·4731 to 1·4759, α_D +13·6 to +18·8°) respectively. These leaf oils were analysed by GLC only (also after fractionation into hydrocarbons and oxygenated terpenes, see below), using a variety of columns to correlate the components with those of red spruce leaf oil. The maximum and minimum values for each recorded component are shown in Fig. 2B, C and D respectively.

Prefractionation

Silicic acid (100 g), deactivated[6,7] with polyethylene glycol (Carbowax 20M, 1 g) was slurried with petrol (b.p. 40–60°) and transferred to a 40 × 4 cm glass column. Red spruce leaf oil (10·0 g) was added on top and washed into the column with three portions (10 ml) of petrol. Elution with petrol (300 ml) gave all the hydrocarbons (2·3 g) as well as a small amount of bornyl acetate (see below). Chloroform (250 ml) was used to elute a mid-fraction (4·5 g) consisting almost entirely of bornyl acetate (98 per cent, n_D 1·4610, α_D −19·07°). Elution with methanol (350 ml) gave the balance of oxygenated terpenes (3·3 g).

Individual Hydrocarbons

The first fraction was divided into C_{10} and C_{15} components by preparative GLC on a 90 × 0·9 cm SE-30 column, which was temperature-programmed from 80 to 150° in 20 min.[6]

The C_{10} hydrocarbons were fractionated on a 600 × 0·9 cm PEG column (100 μl aliquots; 100 to 170° in 40 min) when camphene, β-pinene, and *p*-cymene were isolated practically free from impurities. The isolated α-pinene fraction was found to contain tricyclene (i.r.; r.r.t.). Myrcene was not fully resolved from 3-carene (i.r.; r.r.t.) and the peaks were collected as one fraction. The tail of this fraction was found to contain a little α-phellandrene (u.v.; r.r.t.). Limonene and β-phellandrene (i.r.; u.v.; r.r.t.) were also isolated as a single fraction. Santene (peak 1) and γ-terpinene (peak 12) were identified only by r.r.t. values. Peak 13 appeared to be terpinolene, but no peak with the expected r.r.t. was recorded on the EGPN column.

The C_{15} fraction was resolved similarly on a 300 × 0·9 cm PEG column (30 μl aliquots; 150–205° in 50 min) into four fractions. The first was bornyl acetate (i.r.; r.r.t.) showing that the separation on the silicic acid column was incomplete. Peak 30a could not be correlated with a known sesquiterpene. The i.r. spectrum of this fraction was similar to that of β-caryophyllene, but its r.r.t. differed from that of β-caryophyllene (AN, 0·94; PEG 1·14).

Peak 33a corresponded to bisabolene and peak 34 to a mixture of γ- and δ-cadinene (i.r.; r.r.t.).

The physical constants of the isolated hydrocarbons, including r.r.t. values on four columns are shown in Table 2.

Oxygenated Terpenes

The oxygenated terpenes were fractionated first on a 180 × 0·9 cm SE-30 column to give low boiling (A), middle range (B) and high boiling (C) fractions. From (A) a small amount of 1:8-cineole was isolated on a 180 × 0·4 PEG column. This fraction also contained several minor components of which camphor and linalool were identified (i.r. spectra). The main fraction (B) was too complex to permit isolation of pure components in a single operation and it was found advantageous to subdivide first into mixed alcohols, crude bornyl acetate and crude piperitone fractions on a 180 × 0·9 cm QF-1 column. These fractions were then chromatographed on 180 × 0·4 cm PEG, NGA or Apiezon columns. The components isolated and their physical constants are shown in Table 3. In these experiments a number of new peaks were recorded in small amounts which did not appear to be present in the original oils. It is, therefore, possible that artefacts are formed during repeated fractionation processes. The amounts involved did not exceed 0·1–0·2% of the original oil.

The high boiling fraction (C) was fractionated on a 180 × 0·4 cm PEG column when components 45 and 46, 47, 48 and 56 were obtained in an impure state. Of these components peak 47 was an alcohol which showed tendencies to crystallize. The mixture of components 45 and 46 had an i.r. spectrum which resembled that of farnesyl acetate. Component 48 appeared to be a mixture of an alcohol and acetate and component 56 of an alcohol and a carbonyl compound (1705 cm^{-1}). Although the r.r.t. values indicate C_{15} compounds, it cannot be ruled out that one or more of these components may in fact be C_{10} compounds with an alcoholic and a carbonyl function. Alternatively, these fractions may contain artefacts. Attempts to purify further by GLC on different columns failed to give satisfactory results as decomposition became apparent.

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