Bishomopinolenic (7,11,14-20:3) Acid in Pinaceae Seed Oils

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ABSTRACT: Bishomopinolenic (7,11,14-20:3; BHP) acid has been identified in a sample of pine (*Pinus contorta*) seed oil by gas–liquid chromatography–mass spectrometry of its 4,4-dimethyloxazoline and picolinyl ester derivatives. Neither 20:3n-6 nor 18:3n-6 acids could be detected. The distribution of BHP acid in the seed oils of four conifer families has been established. It only occurred in Pinaceae (*Pinus*, *Abies*, *Cedrus*, *Tsuga*, *Pseudotsuga*, *Larix*, and *Picea*; 72 species analyzed), where it could reach 0.7% of total fatty acids. It could not be detected in Taxaceae (*Taxus baccata*), Cupressaceae (*Juniperus communis*), or Taxodiaceae (*Sciadopytis verticillata*) seed lipids. It is assumed that BHP acid is the elongation product of pinolenic (5,9,12-18:3) acid, and that, at most, 3% of pinolenic acid is elongated to BHP acid. Consequently, the Δ5-desaturation would not necessarily be a final step in the biosynthesis of unsaturated polymethylene-interrupted fatty acids in Pinaceae seeds. Moreover, conifer seeds appear devoid of the Δ6-desaturase.
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KEY WORDS: Bishomopinolenic acid, conifers, 4,4-dimethyloxazoline derivative, mass spectrometry, picolinyl ester, Pinaceae, seed oil.

Δ5-Unsaturated polymethylene-interrupted fatty acids (Δ5-UPIFA, or Δ5-olefinic acids) are seldom present in Angiosperm seed oils, but they are common and characteristic components of Gymnosperm seed lipids (1). These acids are 5,9-18:2 (taxoleic), 5,9,12-18:3 (pinolenic), 5,9,12,15-18:4 (coniferonic), 5,11-20:2, 5,11,14-20:3 (sciadonic), and 5,11,14,17-20:4 (juniperonic) acids (2–6). They are supposed to originate from 9-18:1, 9,12-18:2, and 9,12,15-18:3 acids, and from their elongation products, 11-20:1, 11,14-20:2, and 11,14,17-20:3 acids, through the action of Δ5-desaturase(s) that would act as a final step in their biosynthesis (4). Additionally, a 5,11-18:2 acid, derived from *cis*-vaccenic acid, might be present in *Ginkgo biloba* seed lipids (2).

The fatty acid distribution profiles, and particularly those of Δ5-UPIFA, are so characteristic that they may be used for a chemotaxonomic differentiation of conifer families and, inside Pinaceae, of the principal genera (5). These compositions also allow distinction of some *Pinus* sections (6).

Seed oils of the Pinaceae family generally contain all of the Δ5-olefinic acids, with the exception of juniperonic acid (2–6). In Pinaceae species, the most abundant Δ5-UPIFA, with few exceptions, is pinolenic acid, followed by either taxoleic or sciadonic acids. Coniferonic acid concentration is always low (*ca.* less than 0.1%), whereas 5,11-20:2 acid may reach 1% of total fatty acids.

In addition to Δ5-olefinic acids, and to the common polyunsaturated 9,12-18:2 and 9,12,15-18:3 acids, Pinaceae seed oils contain some 11,14-20:2 acid that is unusual in Angiosperms. According to some authors (7–10), conifer needle and seed lipids would also contain some 20:3n-6 (8,11,14-20:3) acid, which is otherwise characteristic of animal polar lipids. However, the presence of 20:3n-6 acid in conifer lipids is questionable because they do not contain even traces of its precursor, γ-linolenic (6,9,12-18:3) acid. Moreover, the simultaneous presence of both the Δ5-desaturase and the Δ6-desaturase would be exceptional for vegetable species, though this situation was recently observed in a red microalga, *Porphyridium cruentum* (11), and earlier in lower plants, such as mosses and ferns that are able to synthesize arachidonic acid (12).

On the other hand, Ekman (13) has formally identified BHP acid (7,11,14-20:3 in *Picea abies* wood lipids. BHP and 20:3n-6 acids are supposed to elute close to each other during gas–liquid chromatography (GLC) of their methyl esters, and a confusion between both acids might have been possible in earlier studies. We demonstrate here that the component previously identified as 20:3n-6 acid is indeed BHP acid, indicating that in conifer seeds, pinolenic acid may be elongated to BHP acid, at least to a small extent.

EXPERIMENTAL PROCEDURES

Samples. Fatty acid methyl esters (FAME) prepared in duplicate from the seed oil of several conifer species were available from one of us (3–6). The origin of the seeds, the extraction of oils, and the preparation of FAME have been described in detail elsewhere (3–6). 8,11,14-20:3 Acid methyl ester was purchased from the Sigma Chemical Company (St. Louis, MO).

Analytical GLC. FAME were analyzed in a Carlo Erba 4130 chromatograph (Carlo Erba, Milano, Italy), equipped with a DB Wax column (30 m × 0.32 mm i.d., 0.5 μm film; J&W Scientific, Folsom, CA). The oven temperature was 190°C and the inlet pressure of the carrier gas (helium) was 140 kPa. The injector (split mode) and the flame-ionization

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detector were maintained at 250°C. Quantitative data were calculated by an SP 4290 integrator (Spectra Physics, San Jose, CA).

Preparation of 4,4-dimethyloxazoline (DMOX) and picolinyl ester derivatives. DMOX derivatives were prepared directly from the methyl esters by the method of Fay and Richli (14). FAME were hydrolyzed to the free fatty acids (15,16) before conversion to the picolinyl ester derivatives as described by Balazy and Nies (17).

Gas chromatography–mass spectrometry (GC–MS). The derivatives were submitted to GLC–MS with a Hewlett-Packard 5890 Series II plus gas chromatograph, attached to an HP model 5989 MS apparatus (Palo Alto, CA). The latter was used in the electron impact mode at 70 ev with a source temperature of 250°C. The chromatograph was fitted with on-column injection, and equipped with a capillary column of fused silica, coated with BPX-70 [0.22 mm × 50 m; SGE (UK) Ltd., Milton Keynes, United Kingdom]. After holding the temperature at 80°C for 3 min, the column was temperature-programmed at 20°C/min to 160°C, then at 2°C/min to 260°C, where it was held for 5 min. Helium was the carrier gas.

RESULTS AND DISCUSSION

GLC–MS of BHP acid derivatives. The DMOX and picolinyl ester derivatives of the fatty acids were examined by GLC–MS with a highly polar BPX-70 capillary column for the separation. The spectra are illustrated in Figure 1. Both types of derivatives gave prominent molecular ions, which confirmed the chainlength and number of double bonds. The DMOX derivative of 5,9,12-18:3 was known to have an especially prominent ion at *m/z* = 180, representing cleavage between C_7 and C_8, i.e., at the center of the bis-methylene-interrupted ethylenic bond system (14). For a 7,11-double-bond system, the equivalent ion is at *m/z* = 208, and indeed, this was the base peak in the spectrum of the DMOX derivative of 7,11,14-20:3 acid. A gap of 12 amu between *m/z* = 262 and 274 indicated that the third double-bond was in position 14. The spectrum of the picolinyl ester derivative provided absolute confirmation. In this instance, a characteristic ion at *m/z* = 247 represented cleavage between C_9 and C_{10} (c.f., an ion at *m/z* = 219, 28 amu lower, in the mass spectrum of the picolinyl ester derivative of 5,9,12-18:3 acid) and a gap of 26 amu between *m/z* = 300 and 326 confirmed the presence of the double bond in position 14.

Behavior of BHP acid methyl ester during GLC. On the DB Wax capillary column, the BHP acid had an equivalent chainlength (ECL) of 20.93. It emerged just after 5,11,14-20:3 acid (ECL = 20.83), with base-line resolution, whereas an authentic sample of 20:3n-6 acid eluted with exactly the same ECL as BHP acid. As shown in Figure 2, BHP and 20:3n-6 acids, when coinjected, were not resolved on this column and could thus be confused under our operating condi-

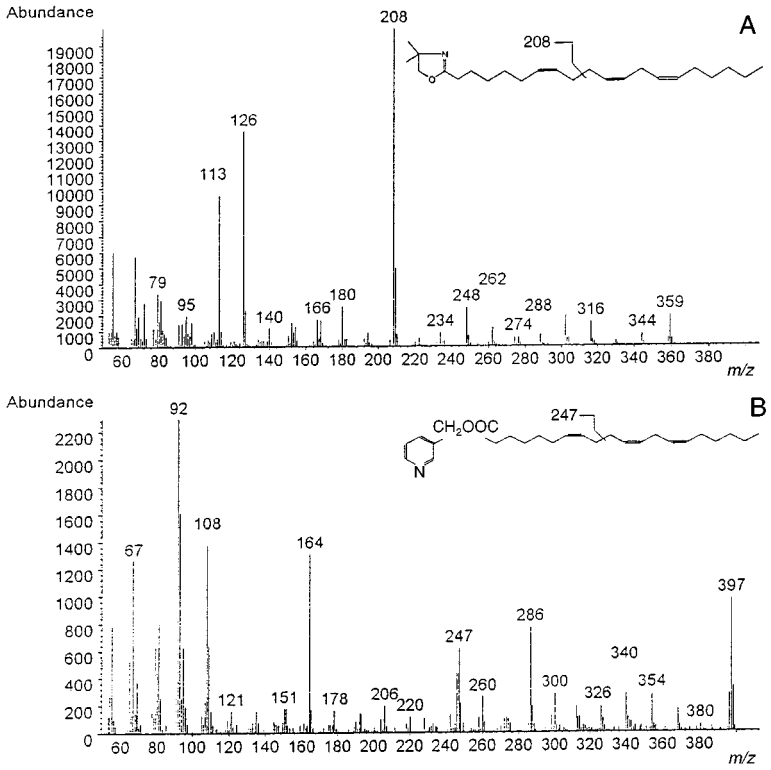

FIG. 1. Mass spectra and fragmentation patterns of the 4,4-dimethyloxazoline (A) and picolinyl ester (B) derivatives of 7,11,14-20:3 (bishomopinolenic) acid prepared from *Pinus contorta* seed oil.

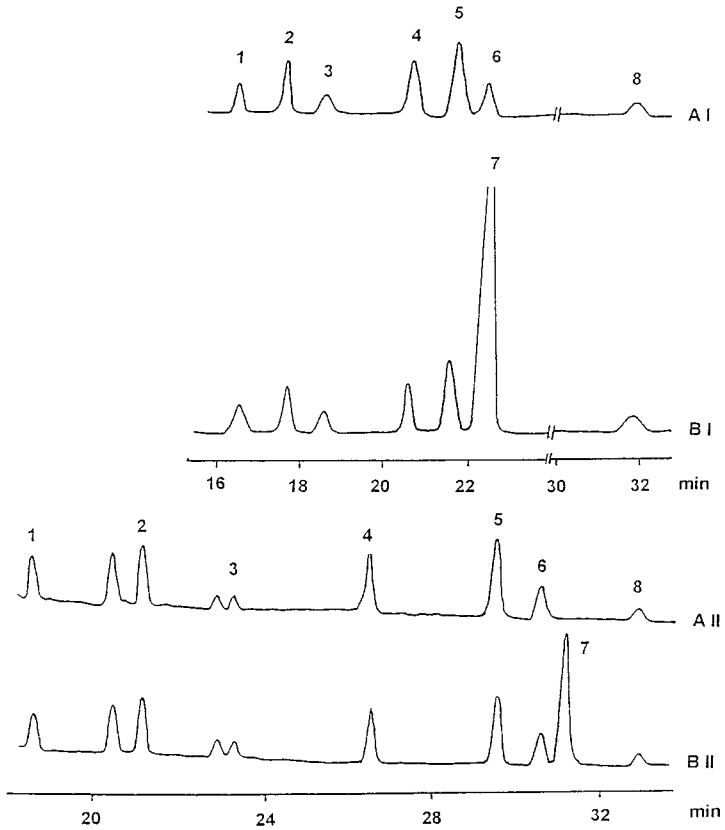

FIG. 2. Partial chromatograms of the 20:0-22:0 region of fatty acid methyl esters prepared with *Pinus contorta* seed oil (A), and of the same preparation spiked with 20:3n-6 acid methyl ester (B). I, analyses on a DB-Wax capillary column (J&W Scientific, Folsom, CA), operated isothermally at 190°C. II, analyses on a CP-Sil 88 capillary column (Chrompack, Middelburg, The Netherlands), operated at 160°C. Identification of peaks: **1**, 20:0; **2**, 11-20:1; **3**, 5,11-20:2; **4**, 11,14-20:2; **5**, 5,11,14-20:3; **6**, 7,11,14-20:3; **7**, 8,11,14-20:3 (added); **8**, 22:0.

tions. On the other hand, 20:3n-6 acid was fully separated from BHP acid on a CP-Sil 88 capillary column (Chrompack, Middelburg, The Netherlands) (Fig. 2). We have verified, for a few Pinaceae species, that no peak was present at the place where 20:3n-6 acid should have eluted on this column.

Moreover, γ-linolenic acid, the precursor of 20:3n-6 acid, could not be detected on chromatograms with either capillary column. On the DB Wax capillary column, γ-linolenic acid had an ECL of 19.01 (4), distinct from that of 5,9,12-18:3 acid (ECL = 18.91). However, owing to the abundance of the latter, 18:3n-6 acid might have been masked by it. Fortunately, this did not occur on a CP-Sil 88 capillary column, where the difference between the ECL of 5,9,12-18:3 and 6,9,12-18:3 acids is 0.18 (4). γ-Linolenic acid could not be observed with this column. This evidenced that conifer seeds are devoid of the Δ6-desaturase, which would have led to the formation of 6,9,12-18:3 acid, the precursor of 8,11,14-20:3 acid.

Distribution of BHP acid in conifer seed oils. Table 1 shows the content of pinolenic and BHP acids in the seed lipids of 72 Pinaceae species. The content of pinolenic acid varied from 0.8% in *Pinus edulis* up to 30.6% in *Larix sibirica*, whereas BHP acid varied from nondetectable amounts up to 0.7% (*Pinus contorta*). Generally, BHP acid was either nondetectable or low in those species for which the content of pinolenic was low (*ca*. equal to or less than 11%). From data in Table 1, it can be estimated that the elongation product of pinolenic acid (BHP acid) is at most equal to 3%, but generally less than 2%. BHP acid could not be detected in the seed lipids from *Taxus baccata*, *Juniperus communis*, and *Sciadopytis verticillata*, each of which being representative of Taxaceae, Cupressaceae, and Taxodiacea families, respectively (results not shown). This is not surprising because pinolenic acid in species from these families is less than 3.4% of total fatty acids (2,4,5). Thus, even if pinolenic acid is elongated in these families at the same rate as in Pinaceae, BHP acid would be too low to be detected on chromatograms.

This study has demonstrated that BHP acid is present in most Pinaceae species analyzed. This indicates that pinolenic acid is elongated to a small extent and, consequently, that Δ5-desaturation is not necessarily a terminal step in the biosynthesis of unsaturated polymethylene-interrupted fatty acids in conifers, particularly in Pinaceae. In other plant seeds, a Δ7-ethylenic bond has only been observed in some genera of the Lamiaceae, subfamily Lamiodeae [7,8-20:2 (phlomic) acid, the elongation product of 5,6-18:2 (laballenic) acid] (18). Moreover, our study shows that earlier reports on 20:3n-6 acid occurring in conifer lipids (7–10) were probably erroneous.

TABLE 1
Pinolenic Acid (PA) and Bishomopinolenic Acid (BHPA) Content of Pinaceae Seed Lipids (wt% of total fatty acids, mean)

Species	PA	BHPA	Species	PA	BHPA	Species	PA	BHPA
Pinus banksiana	22.90	0.56	*P. laricio*	19.44	0.30	*P. abies*	24.67	0.16
P. contorta	22.75	0.68	*P. koekelare*	18.97	0.35	*P. sitchensis*	25.75	0.16
P. palustris	19.93	0.34	*P. nigra*	18.76	0.32	*P. glauca*	24.06	0.13
P. elliotii	18.47	0.37	*P. massoniana*	18.20	0.14	*Cedrus atlantica*	10.54	0.24
P. caribaea	17.71	0.33	*P. mughus*	19.55	0.36	*C. atlantica glauca*	9.94	0.20
P. echinata	18.18	0.28	*P. cembra*	19.19	0.14	*C. libani*	10.50	0.15
P. occidentalis	18.50	0.22	*P. peuce*	25.18	0.30	*C. deodara*	9.61	0.16
P. attenuata	20.37	0.32	*P. monticola*	20.83	0.21	*C. brevifolia*	10.74	0.34
P. muricata	22.80	0.46	*P. parviflora*	18.18	0.13	*Abies lasiocarpa*	17.11	0.09
P. radiata	22.34	0.27	*P. strobus*	25.29	0.47	*A. pinsapo*	10.39	0.08
P. taeda	18.34	0.32	*P. sibirica*	18.49	0.10	*A. balsamea*	14.94	0.14
P. pinaster	7.90	0.18	*P. koraiensis*	14.52	trace	*A. cephalonica*	11.94	0.07
P. ponderosa	18.36	0.20	*P. griffithii*	21.69	0.16	*A. fraserii*	14.04	0.12
P. michoacana	18.76	0.24	*P. canariensis*	1.49	—[b]	*A. equi-trojani*	11.83	0.11
P. jeffreyi	11.28	trace[a]	*P. pinea*	0.35	—	*A. nordmanniana*	11.37	0.09
P. halepensis	4.02	0.04	*P. aristata*	12.44	trace	*A. bornmulleriana*	11.42	0.10
P. brutia	3.14	0.04	*P. edulis*	0.36	—	*A. alba*	12.47	0.10
P. eldarica	4.42	trace	*Tsuga canadensis*	19.53	0.41	*A. concolor*	8.78	0.09
P. sylvestris	21.65	0.65	*T. heterophylla*	24.11	0.13	*A. grandis*	11.15	—
P. resinosa	21.09	0.20	*Pseudotsuga menziesii*	18.37	0.11	*A. nobilis*	11.15	0.14
P. uncinata	20.44	0.42	*Picea engelmanii*	24.89	0.17	*A. lowiana*	13.32	0.07
P. thunbergii	17.80	0.21	*P. pungens glauca*	24.59	0.12	*Larix leptolepis*	25.81	0.22
P. salzmannii	19.54	0.31	*P. omorika*	25.02	0.17	*L. decidua*	28.21	0.29
P. pumilia	18.74	0.32	*P. orientalis*	23.54	0.16	*L. sibirica*	30.57	0.34

[a]Trace amounts; peaks visible on the chromatogram, but not taken into account by the integrator.
[b]Not detected.

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