Chemical Evaluation of Volatile Oils in Eucalyptus Species

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The volatile leaf oils of *Eucalyptus kochii* subsp. *kochii*, *E. kochii* subsp. *plenissima*, and *Eucalyptus oleosa* var. borealis, potential Western Australian candidates for commercial eucalyptus oil production, were analyzed by means of GC, GC/MS, and IR. The three taxa were found to produce similar but distinctive individual distributions of leaf oil terpenoids. This supports their close botanical relationship. The oils consist mainly of monoterpenoids, with 1,8-cineole dominating to the extent of 80–90%. Approximately 55 other components were detected. The oils of *Eucalyptus globulus* and *Eucalyptus kondininensis* were studied also.

The leaf oils of eucalypts are now of commercial importance in many countries but the eucalyptus species originated in Australia. Guenther (1950) provides a very comprehensive review of the analysis of eucalyptus oils in Australia. It traces the origin of the first export from Port Jackson, Sydney, in 1895 (the Sydney peppermint) and discusses the various commercial species, distillation techniques, and the many applications of the oil. Pioneering efforts have been made by Bosisto, Baker and Smith (1920), and Penfold and Willis (1961), and the current situation has been discribed by Small (1981), Lassak (1988), and Beckmann (1988). Nearly all eucalypts have glands in their leaves that produce oils giving them their characteristic odors. However, the yields and chemical compositions of the leaf oil vary widely, both between species and between individuals. The oils contain mainly monoterpenoids and small amounts of sesquiterpenoids. Approximately 70–85% of the oil of *Eucalyptus polybractea* and *Eucalyptus radiata*, current commercial species, comprises cineole (1,8-cineole; 1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane; $C_{10}H_{18}O$). This differs from China eucalyptus oil derived from the camphor industry as it contains 60–75% 1,8-cineole, monoterpene hydrocarbons, small amounts of 1,4-cineole and camphor, but no sesiquiterpenoids.

Pharmaceutical-grade eucalyptus oil as defined in most standards requires a minimum cineole level of 70%. Although cineole has a wide distribution in a large number of plant species, and can be obtained synthetically, eucalyptus oil remains the most important source (Tjandra, 1986).

In the search for higher oil yielding species and individuals, large numbers of eucalyptus leaf samples have to be analyzed. Since the existing steam distillation method for the extraction and analysis is impractical and time-consuming, a rapid, simple, and sensitive solvent extraction method described elsewhere (Ammon et al., 1985a,b) was developed, involving solvent extraction of the leaf material with ethanol in the field, followed by laboratory water and oil analyses of the foliage extract using both Karl-Fischer titrations and gas–liquid chromatography. This paper presents the results of a study of the chemical composition and variability of several high oil producing Western Australian eucalypts carried out as part of a research project that also investigated the use of eucalyptus oil as a liquid fuel component (Ammon et al., 1986; Barton and Tjandra, 1988, 1989).

EXPERIMENTAL SECTION

Gas–Liquid Chromatography (GLC). Gas–liquid chromatography was carried out on a Hewlett-Packard 5790 gas chromatograph equipped with a FID detector. The chromatograph was fitted with a 25 mm × 0.31 mm (i.d.) fused silica capillary column, coated with cross-linked 5% phenylmethylsilicone. The column temperature was initially 80 °C, was held for 5 min, and was programmed to rise from 80 to 180 °C at 5 °C/min. The maximum temperature was maintained for a further 7.5 min before cooling. The total analysis time was thus 32.5 min. The injection and detector temperatures were 250 and 300 °C, respectively. Helium was used as the carrier gas with a flow rate of 2.25 mL/min. The splitting ratio was 63:1. Air and hydrogen flow rates were 240 and 27 mL/min, respectively. The sample size (solution in ethanol) was 1 μL. The chromatograms were recorded and processed by Hewlett-Packard 3390 A integrator.

GC/MS. A Hewlett-Packard 5986 gas chromatograph/mass spectrometry system equipped with an OV-101 0.31 mm (i.d.) × 25 m WCOT capillary column was directly coupled to the ion source of the MS. The MS was electron ionization, ion source 200 °C, at 35 eV. Compound identifications were based on published spectra, comparison with reference compounds, and interpretation of MS fragmentation patterns.

IR. A Pye Unicam infrared spectrometer, Model SP2000, was used. Infrared spectra of liquid compounds were obtained from thin films sandwiched between potassium bromide disks, while those of solid compounds were obtained with use of the potassium bromide disk technique. All spectra were recorded in the 4000–200-cm^{-1} region. All controls were set to normal positions, and the scan time was 7 min.

NMR. Bruker (90-MHz) and Hitachi (60-MHz) NMR spectrometers were used. All chemical shifts are reported with reference to tetramethylsilane at δ 0.00.

Fractionation of Oxygenated Components from Terpene Hydrocarbons. Liquid–solid chromatography (LSC) was carried out on a 50 cm × 18 mm (i.d.) column. The method of Rockland and Debenedict (1975), which involves elution of hydrocarbons with pentane and oxygenated compounds with ethyl acetate from alumina pretreated with ethyl acetate, was used to increase the concentration of minor components for identification by GC/MS. The obvious change in the refractive index of the eluate and the appearance of a pale yellow band at about the same time indicated the initial elution of the oxygenated compounds.

RESULTS AND DISCUSSION

***Eucalyptus kochii* and *Eucalyptus oleosa*.** The leaf oils of *E. kochii* subsp. *kochii*, *E. kochii* subsp. *plenissima* (Brooker et al., 1988), and *E. oleosa* var. borealis obtained from different locations in Western Australia were analyzed for their chemical constituents. The species were found to produce remarkably consistent individual distinctive distribution patterns of leaf oil terpenoids. The oils contain mainly monoterpenoids, with 1,8-cineole dominating to the extent of 80–90% (see Tables I–III). Approximately 55 components were detected, the more significant being 3-methylbutanal, α-thujene, α-pinene, *p*-cymene, γ-terpinene, terpinolene, linalool, *trans*-pinocarveol, citronellal, borneol, terpinen-4-ol, β-terpineol, α-terpineol, verbenone, cuminal, carvone, neral, terpinyl acetate, β-caryophyllene, aromadendrene, and globulol.

Figures 1–3 present the typical chromatograms of the oils of these three morphologically similar taxa. Each

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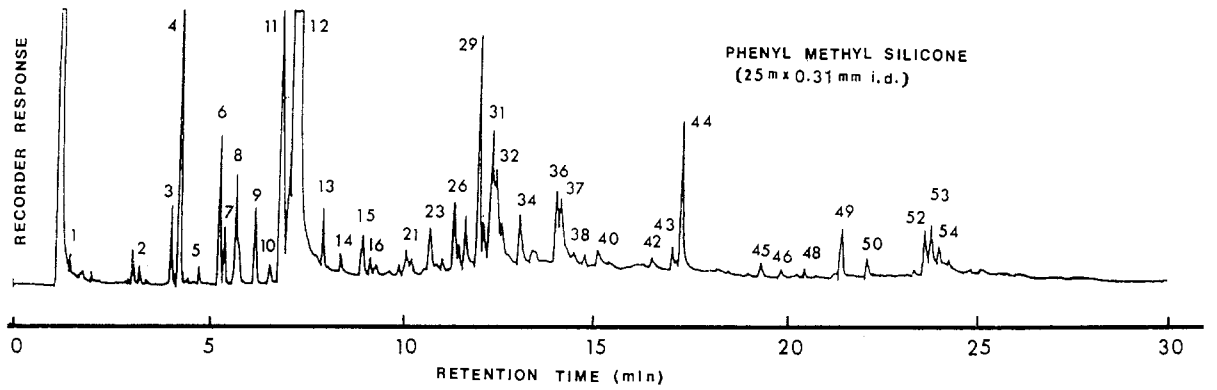

Figure 1. GLC chromatogram of the leaf oil of *E. kochii* (see key to components in Table I).

Table I. Chemical Constituents of the Volatile Oils of *E. kochii* subsp. *kochii* (See Figure 1)

peak	compound	% compn in oil[f]
1	3-methylbutanal[a,c]	tr
2	unknown	tr
3	α-thujene[a,b]	tr
4	α-pinene[a-c]	1.5 ± 0.5
5	camphene[a,b]	tr
6	sabinene[a,b]	tr
7	β-pinene[a,b]	0.6 ± 0.3
8	β-myrcene[a]	tr
9	α-phellandrene[a]	tr
10	α-terpinene[a]	tr
11	*p*-cymene[a-c]	1.5 ± 0.4
12	1,8-cineole[a-d]	80.5 ± 3.2
13	γ-terpinene[a]	tr
14	octan-1-ol[e]	tr
15	terpinolene[a,c]	tr
16	unknown	tr
17	linalool[a]	tr
18	fenchol[e]	tr
19	unknown	tr
23	*trans*-pinocarveol[e]	tr
25	citronellal[a]	tr
26	isoborneol[a]	tr
27	unknown	tr
28	borneol[a]	tr
29	terpinen-4-ol[a]	0.7 ± 0.3
31	cryptone[e]	tr
32	β-terpineol[a,b]	0.5 ± 0.2
33	α-terpineol[a,b]	tr
34	verbenone[a]	tr
36	cuminal[a]	0.6 ± 0.2
37	carvone[a]	tr
39	neral[a]	tr
44	terpinyl acetate[a]	tr
45	β-caryophyllene[a-c]	tr
46	aromadendrene[a-c]	tr
49	eremophilene[e]	tr
50	β-sesquiphellandrene[e]	tr
53	globulol[a-c]	tr

[a] From comparisons of GLC retention data with those of standard compounds on columns of different polarity. [b] Mass spectrum consistent with published data and interpretation. [c] IR. [d] ^{1}H NMR. [e] Tentative. [f] tr = trace (less than 0.3%); values are the means of 30 individual trees.

Table II. Chemical Constituents of the Volatile Oils of *E. kochii* subsp. *plenissima* (See Figure 2)

peak	compound	% compn in oil[f]
1	3-methylbutanal[a,c]	tr
2	unknown	tr
3	α-thujene[a,b]	tr
4	α-pinene[a,b]	1.8 ± 0.5
5	camphene[a,b]	tr
6	sabinene[a,b]	tr
7	β-pinene[a,b]	0.6 ± 0.3
8	β-myrcene[a]	tr
9	α-phellandrene[a]	tr
10	α-terpinene[a]	tr
11	*p*-cymene[a-c]	1.5 ± 0.3
12	1,8-cineole[a-d]	77.3 ± 4.5
13	γ-terpinene[a]	tr
14	octan-1-ol[e]	tr
15	terpineolene[a,c]	tr
16	unknown	tr
17	linalool[a]	tr
18	fenchol[e]	tr
19	unknown	tr
23	*trans*-pinocarveol[e]	tr
25	citronellal[a]	tr
26	isoborneol[a]	tr
27	unknown	tr
28	borneol[a]	tr
29	terpinen-4-ol[a]	0.7 ± 0.3
31	cryptone[e]	tr
32	β-terpineol[a,b]	0.5 ± 0.2
33	α-terpineol[a,b]	tr
34	verbenone[a]	tr
36	cuminal[a]	0.6 ± 0.2
37	carvone[a]	tr
39	neral[a]	tr
44	terpinyl acetate[a]	tr
45	β-caryophyllene[a,c]	tr
46	aromadendrene[a-c]	tr
49	eremophilene[e]	tr
50	β-sesquiphellandrene[e]	tr
53	globulol[a-c]	tr

[a] From comparisons of GLC retention data with those of standard compounds on columns of different polarity. [b] Mass spectrum consistent with published data and interpretation. [c] IR. [d] ^{1}H NMR. [e] Tentative. [f] tr = trace (less than 0.3%); values are the means of 30 individual trees.

chromatogram differs only slightly from the others, thus lending support to this close taxonomic relationship.

The enrichment of the compounds in the various fractions obtained by LSC facilitated GC and GC/MS identification on a number of minor constituents. In this manner the presence of α-thujene, α-terpinene, γ-terpinene, *d*-limonene, terpinolene, α-terpineol, verbenone, terpinyl acetate, aromadendrene, and globulol in the leaf oil of eucalypts was established.

Several studies on the mechanism of genetic inheritance of the terpenoids have been reported (Banthrope et al., 1972), but many aspects remain uncertain. Experimental results on the studied species indicated that ecological differences did not affect the qualitative leaf oil composition. There was a seasonal variation in 1,8-cineole yields (Brooker et al., 1988), but the components of the oil showed few differences. Therefore, it can be said that there was a quantitative, but not qualitative, change in the oil composition. Samples from an experimental seedling plantation produced the same general leaf oil compositions as the parent populations (Tjandra, 1986). Therefore, it is believed that chemical constituents of leaf oil compo-

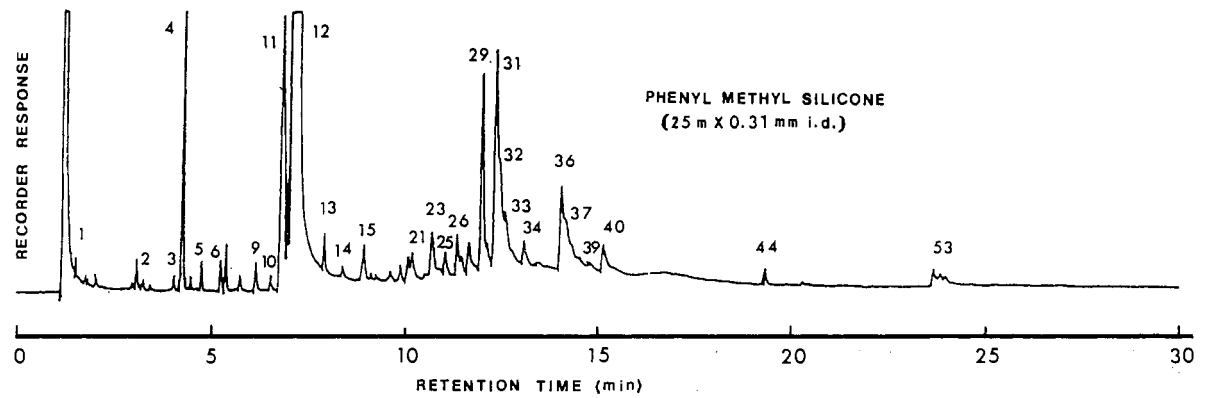

Figure 2. GLC chromatogram of the leaf oil of *E. kochii* subsp. *plenissima* (see key to components in Table II).

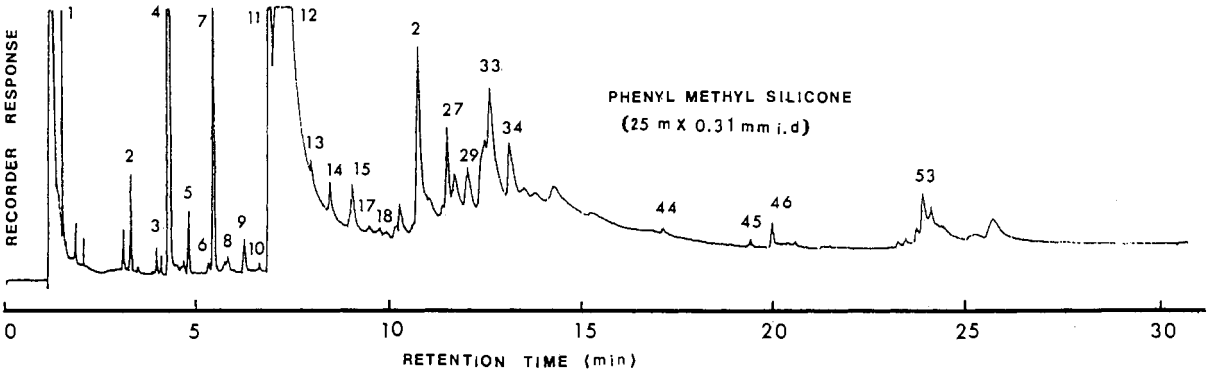

Figure 3. GLC chromatogram of the leaf oil of *E. oleosa* var. borealis (see key to components in Table III).

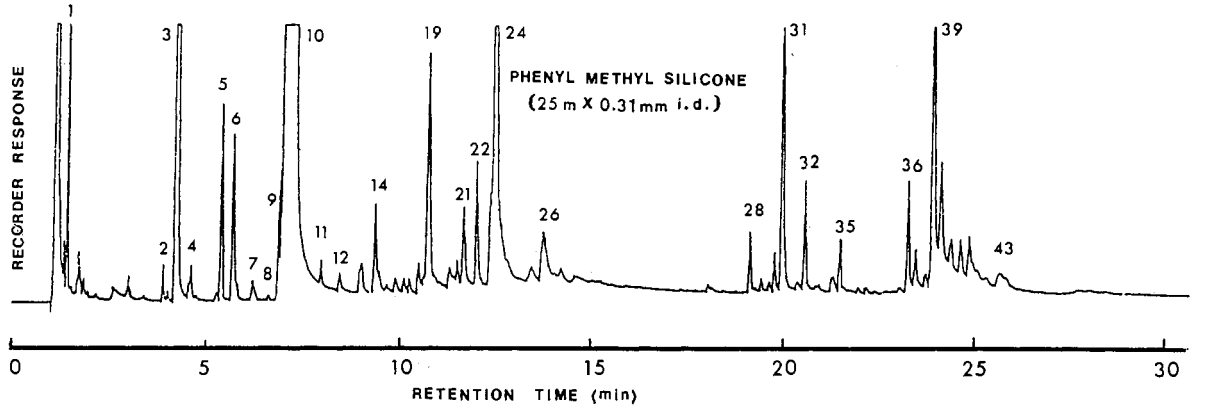

Figure 4. GLC chromatogram of the leaf oil of *E. globulus* (see key to components in Table IV).

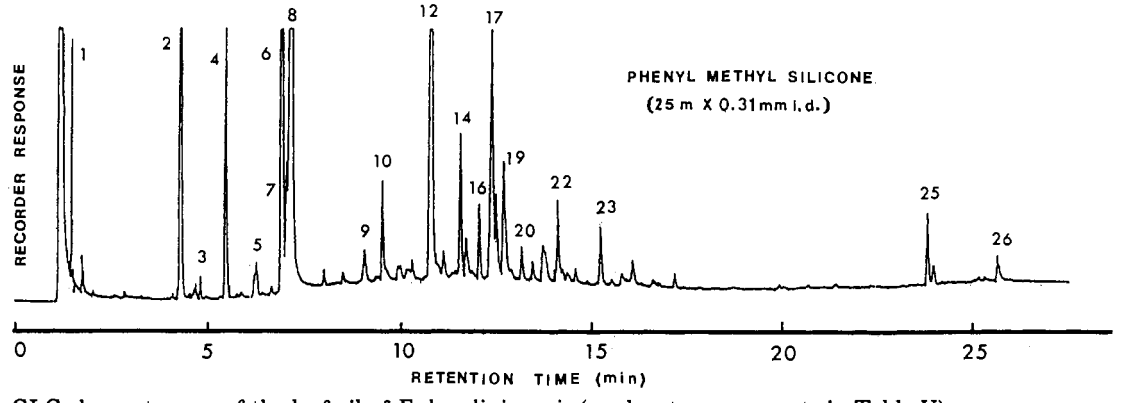

Figure 5. GLC chromatogram of the leaf oil of *E. kondininensis* (see key to components in Table V).

sitions result from genetic inheritance. In this respect, chemical data on leaf oil composition may provide additional information for the clarification of long-standing classification problems.

Eucalyptus globulus. *E. globulus* Labill. (Tasmanian blue gum), now perhaps the most widely distributed eucalypt in the world, is the main commercial source of essential oil and pulp wood in many countries (Nishimura

Table III. Chemical Constituents of the Volatile Oils of *E. oleosa* var. borealis (See Figure 3)

peak	compound	% compn in oil[f]
1	3-methylbutanal[a,c]	tr
2	unknown	tr
3	α-thujene[a,b]	tr
4	α-pinene[a,b]	2.0 ± 0.6
5	camphene[a,b]	tr
6	sabinene[a,b]	tr
7	β-pinene[a,b]	1.0 ± 0.3
8	β-myrcene[a]	tr
9	α-phellandrene[a]	tr
10	α-terpinene[a]	tr
11	*p*-cymene[a-c]	2.5 ± 0.7
12	1,8-cineole[a-d]	75.5 ± 0.7
13	γ-terpinene[a]	tr
14	octan-1-ol[e]	tr
15	terpinolene[a,c]	tr
16	unknown	tr
17	fenchol[e]	tr
18	fenchol[e]	tr
19	unknown	tr
23	*trans*-pinocarveol[e]	0.7 ± 0.4
25	citronellal[a]	tr
26	isoborneol[a]	tr
27	unknown	tr
28	borneol[a]	tr
29	terpinen-4-ol[a]	tr
31	cryptone[e]	tr
32	β-terpineol[a,b]	0.5 ± 0.2
33	α-terpineol[a,b]	0.5 ± 0.2
34	verbenone[a]	tr
36	cuminal[a]	tr
37	carvone[a]	tr
39	neral[a]	tr
44	terpinyl acetate[a]	tr
45	β-caryophyllene[a,c]	tr
46	aromadendrene[a-c]	tr
49	eremophilene[e]	tr
50	β-sesquiphellandrene[e]	tr
53	globulol[a-c]	tr

[a] From comparisons of GLC retention data with those of standard compounds on columns of different polarity. [b] Mass spectrum consistent with published data and interpretation. [c] IR. [d] ^{1}H NMR. [e] Tentative. [f] tr = trace (less than 0.3%); values are the means of 30 individual trees.

and Calvin, 1979). The leaves yield about 1–2% on a dry weight basis of oil containing 60–70% cineole. The oil of this species differs from that of the species discussed earlier as illustrated in Figure 4 and Table IV, consisting mainly of monoterpenoids and sesquiterpenoids, the two most significant sesquiterpenoids being peaks 31 and 39 in the chromatogram, aromadendrene and globulol.

Eucalyptus kondininensis. Although 1,8-cineole can be obtained synthetically (Aikawa et al., 1965; Coxon et al., 1968; Matsubara et al., 1968; Davis, 1975; Goldstein, 1982), the most important source will probably remain the naturally occurring eucalypts. As the use of eucalyptus oil and cineole broadens, it is appropriate to explore potential new sources of oil. In view of the vast areas of salty, arid regions available in Australia, it is desirable to cultivate plantations of high oil yield and salt-tolerant flora. *E. kondininensis* (2–4% oil on a dry weight basis) is suitable for extreme saline conditions (see Figure 5 and Table V).

CONCLUSION

The large variation in terpenoid compositions found in the eucalypts makes great demands on analytical techniques for their resolution and identification. The existence of the chemical varieties indicates the importance of selective cultivation to ensure the product meets the specific industrial or commercial demand.

Table IV. Chemical Constituents of the Volatile Oils of *E. globulus* (See Figure 4)

peak	compound
1	3-methylbutanal[a,c]
2	α-thujene[a,b]
3	α-pinene[a-c]
4	camphene[a,b]
5	β-pinene[a,b]
6	β-myrcene[a]
7	α-phellandrene[a]
8	α-terpinene[a]
9	*d*-limonene[a,b]
10	1,8-cineole[a-d]
11	γ-terpinene
12	octan-1-ol[e]
14	linalool[a]
15	fenchol[a]
19	*trans*-pinocarveol[e]
21	borneol[a]
22	terpin-4-ol[e]
23	isomenthol[e]
24	β-terpineol[a,b]
29	*trans*-caryophyllene[a,c]
30	δ-gurjunene[e]
31	aromadendrene[a-d]
32	alloaromadendrene[e]
35	eremophilene[e]
39	globulol[a-c]

[a] From comparisons of GLC retention data with those of standard compounds on columns of different polarity. [b] Mass spectrum consistent with published data and interpretation. [c] IR. [d] ^{1}H NMR. [e] Tentative.

Table V. Chemical Constituents of the Volatile Oils of *E. kondinensis* (See Figure 5)

peak	compound
1	3-methylbutanol[a,c]
2	α-pinene[a-c]
3	camphene[a,b]
4	β-pinene[a,b]
5	α-phellandrene[a]
6	*p*-cymene[a-c]
7	*d*-limonene[a-c]
8	1,8-cineole[a-d]
9	terpinolene[a,c]
10	unknown
12	*trans*-pinocarveol[e]
14	unknown
15	borneol[a]
16	terpin-4-ol[a]
17	cryptone[e]
18	β-terpineol[a,b]
19	α-terpineol[a,b]
20	verbenone[a]
22	cuminal[a]
23	neral[a]
26	β-eudesmol[a]

[a] From comparisons of GLC retention data with those of standard compounds on columns of different polarity. [b] Mass spectrum consistent with published data and interpretation. [c] IR. [d] ^{1}H NMR. [e] Tentative.

ACKNOWLEDGMENT

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Registry No. 3-Methylbutanal, 590-86-3; α-thujene, 2867-05-2; α-pinene, 80-56-8; camphene, 79-92-5; sabinene, 3387-41-5; β-pinene, 127-91-3; β-myrcene, 123-35-3; α-phellandrene, 99-83-2; α-terpinene, 99-86-5; *p*-cymene, 99-87-6; 1,8-cineole, 470-82-6; γ-terpinene, 99-85-4; octan-1-ol, 111-87-5; terpinolene, 586-62-9; linalool, 78-70-6; fenchol, 1632-73-1; *trans*-pinocarveol, 1674-08-4;

citronellal, 106-23-0; isoborneol, 124-76-5; borneol, 507-70-0; terpinen-4-ol, 562-74-3; cryptone, 500-02-7; β-terpineol, 138-87-4; α-terpineol, 10482-56-1; verbenone, 18309-32-5; cuminal, 122-03-2; carvone, 99-49-0; neral, 106-26-3; terpinyl acetate, 8007-35-0; β-caryophyllene, 87-44-5; aromadendrene, 72747-25-2; eremophilene, 10219-75-7; β-sesquiphellandrene, 20307-83-9; globulol, 489-41-8; *d*-limonene, 5989-27-5; terpin-4-ol, 80-53-5; isomenthol, 490-99-3; *trans*-caryophyllene, 87-44-5; alloaromadendrene, 25246-27-9; β-eudesmol, 473-15-4.

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Optimization of Enzymatic Phosphorylation of Soybean Storage Proteins: Glycinin and β-Conglycinin

Lynne F. Ross

Enzymatic phosphorylation could provide a means of increasing the solubility of soy proteins at mildly acidic pH and thus extending the availability of soy proteins for use in food systems. Soybean storage proteins, glycinin and β-conglycinin, were enzymatically phosphorylated with a commercial preparation of the catalytic subunit of cAMP-dependent protein kinase isolated from bovine cardiac muscle. The method was optimized for soybean protein substrates. The degree of phosphorylation was increased 150-fold, from 50 pmol of phosphate/mg of soy protein to 8.9 nmol of phosphate/mg of total soy protein. Soy flours and isolates incorporated up to 1.24 mol of phosphate/mol of β-conglycinin and over 2.0 mol of phosphorus/mol of glycinin.

Due to their high protein content, soybean flours, concentrates, and isolates are gaining importance in food systems. These proteins have good functional properties, such as high solubility and emulsifying ability, except in the acidic range since solubility decreases in this range due to the isoelectric point of most soy proteins. Therefore, these proteins cannot be used in acid foods such as beverages, coffee whiteners, or mayonnaise.

Phosphorylation increases solubility and emulsifying ability of soybean proteins, particularly in the acidic range (Sung et al., 1983; Hirotsuka et al., 1984). In previous experiments (Ross and Bhatnagar, 1989) a homogeneous preparation of the catalytic subunit of cAMP-dependent protein kinase (cAMPdPK) with high specific activity (12.6 μmol of γ-^{32}P transferred/min per mg of Ser peptide) was found to phosphorylate soybean storage proteins, glycinin and β-conglycinin. With the homogeneous enzyme preparation, protein from soy flour incorporated 50 pmol of phosphate/mg of soy protein and protein from soy isolates incorporated from 2.85 to 7.58 nmol of phosphate/mg of protein. Since the ultimate aim of this research is to use this method of phosphorylation in the food processing industry, the commercially available enzyme preparation

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