during the early stage of heating. Further studies are necessary to ascertain the structure of products responsible for the antioxidative properties of heated phospholipids.

From the foregoing discussion, it can be concluded that polar browning reaction products affect oil stability when oil is heated in the presence of phospholipids.

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High Oil- and Polyphenol-Producing Species of the Northwest

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The examination of plant species for their potential as renewable sources of industrial raw materials, conducted at the Northern Regional Research Center, has been extended to include 110 species from North Dakota (ND), Colorado (CO), and Oregon (OR), U.S.A. Plant samples were collected and analyzed for yields of "oil," "polyphenol," "hydrocarbon" and crude protein as well as for botanical characteristics. Data are presented only for the relatively high-yielding species. Oil and hydrocarbon extracts of plants that yielded at least 3.0% oil (dry, ash-free, plant sample basis) and/or at least 0.4% hydrocarbon were analyzed for classes of constituents. Oils of such species were saponified to determine yields of fatty acids and unsaponifiable matter. Hydrocarbon was examined for the presence of rubber, gutta and/or waxes. Polyisoprenes were analyzed for average molecular weight and molecular weight distribution. Even when compared to about 1000 species previously analyzed in this program, seven of the species yielded high amounts of oil (7.1-11.1%) plus substantial amounts of polyphenol (10.0-19.7%). Of these, six are evergreen trees or shrubs and one is a nonwoody perennial. Another seven species yielded substantial amounts of oil (5.4-6.6%), of which five gave 17.1-24.7% polyphenol. The most notable oil-producing species were *Juniperus scopulorum* (11.1%), *Pinus albicaulis* (10.1%), *Pinus flexilis* (9.3%), *Pinus mugo* (8.4%), *Liatris punctata* (8.0%) and *Juniperus communis* (7.8%). Crude protein contents for all 22 species were low (4.2%) to moderate (10.4%). Maximum hydrocarbon content for the 22 selected species reported was only 0.5%. The highest total amount of oil, polyphenol, hydrocarbon and crude protein was 38.9% for *Acer ginnala*. Data obtained in this study are discussed with respect to those from species previously analyzed in this program.

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Development of new crops for production on underused land could stimulate industrial and economic growth without competing with established crops (1-4). Currently, U.S. food crops are much in excess of domestic needs. New nonfood crop developments could reduce our nation's dependency upon foreign sources of essential and strategic materials as well as have numerous other benefits (1,4).

In 1974, the Northern Regional Research Center (NRRC) began a program to study "whole-plant" species in efforts to identify potential new-crop candidates for the production of fuels, chemicals and other industrial raw materials (5). About 500 species were analyzed in this program between 1974 and 1982 (5-8). Since early1983 some 700 additional species from various regions of the U.S. have been studied (9-14).

Recently, we have reported on high oil-bearing species collected from Arizona (10-12). Our present study of 110 species collected from ND, CO and OR includes many species with high quantities of solvent-extractable fractions referred to as "oils" and "polyphenols." Most of such species were evergreen trees and shrubs containing little "hydrocarbon." The terms "oil," "polyphenol," "hydrocarbon," "whole-plant" and other terms are qualified in this report and have been discussed previously (5,8,9,12). A checklist (without chemical data) of the first 500 species examined has been published (7), and a checklist of the subsequent species examined, including the most recent 110, is forthcoming. As in earlier reports (5-14), data are presented for selected species yielding relatively high quantities of oil, polyphenol, hydrocarbon and/or proteins. However, analytical data for all species are available from this Center.

EXPERIMENTAL PROCEDURES

North Dakota (ND) plant samples (60 species) were collected by Gordon A. Hensen, Research Technician, Northern Great Plains Research Center, USDA-ARS, Mandan, North Dakota. Colorado (CO) samples (30 species) were collected by Dale W. Funk and Nanette E. Moss, graduate students under Samuel Shushan, Department of Environmental, Population and Organismic Biology, University of Colorado, Boulder, Colorado. Oregon (OR) samples (20 species) were collected by Steven Broich, a student under Kenton L. Chambers, Department of Botany, Oregon State University, Corvallis, Oregon. Voucher specimens of all species were prepared by the collectors and are filed at the Northern Regional Research Center herbarium.

Herbaceous plants were collected as mature plants, clipped at ground level. Samples from trees and large shrubs were collected by removing the latest 1-3 years' growth, including the stems and leaves as well as any fruits and seeds remaining on the plant samples. Although such samples have been referred to as "whole-plant" samples (5,8), we refer simply to "plant" samples in this report. The plant samples were allowed to dry in a sheltered area at ambient conditions (15-30 C) in ND, CO and OR. The entire quantity of each sample (about 500-1000 g, dry basis) was ground in a Wiley-type mill equipped with a screen containing 1 mm-diameter holes. Subsamples of milled material were analyzed for moisture (volatiles), ash and apparent crude protein (6.25 × % Kjeldahl nitrogen). Each milled sample (about 100 g) was extracted in a Soxhlet apparatus with acetone for 48 hr, after which acetone was evaporated using a stream of filtered air. The air-dried extract was partitioned between hexane and water:ethanol (1:7) to obtain fractions referred to as "oil" and "polyphenol," respectively. These fractions were oven-dried (105 C, 2 hr) and weighed for yield. After the 48-hr acetone extraction, each plant sample was extracted with hexane for 48 hr to obtain a fraction referred to as "hydrocarbon." After the hexane was evaporated with a stream of filtered air, the hydrocarbon was oven dried (105 C, 2 hr) and weighed for yield.

If yield of oil was at least 3.0% on a moisture-free plus ash-free plant sample basis, the oil was analyzed by thin layer chromatography (TLC). Each oil sample (1 g) was mixed with Darco S-51 activated carbon (1 g), Celite (1 g), and hexane (200 ml). The mixture was warmed over a steam bath for 10 min and filtered through Whatman No. 2 filter paper. The filtered oil was spotted adjacent to a standard reference mixture of sitosterol, oleyl alcohol, oleic acid, triolein, oleyl laurate, and squalene on TLC plates (Silica Gel 60, 0.25 mm thick layer). Chromatograms were developed with hexane:diethyl ether: acetic acid (80:20:1) and then dried, sprayed with a 40% sulfuric acid-5% potassium dichromate solution, and charred at about 200 C. Oils were saponified by conventional procedures (15), and their constituents were partitioned between 50% aqueous ethanol and hexane to obtain sodium salts of organic acids in aqueous ethanol and unsaponifiable matter in the hexane. The aqueous alcohol portions were acidified and extracted with hexane to obtain the organic acids. Free organic acids and unsaponifiable matter were oven-dried (105 C, 2 hr) and weighed for yield.

If yield of hydrocarbon extract was at least 0.4% hydrocarbon films on sodium chloride discs were examined for the presence of rubber, gutta and waxes using a Perkin Elmer, Model 137, Spectrophotometer. Weight-average molecular weight (MW) and molecular weight distribution (MWD) of rubber and gutta, dissolved in tetrahydrofuran, were determined by gel permeation chromatography on a Waters Model ALC/GPC 244 Liquid Chromatograph using polystyrene standards (16).

RESULTS AND DISCUSSION

Table 1 presents some general information for selected species of the 110 examined. Sixteen of the 22 species listed are evergreen trees or shrubs. Three species are deciduous shrubs and three are perennial herbs. Samples of 17 species listed were from Colorado. No Oregon species of the 20 collected are represented because yields of acetone- and hexane-extractable constituents were low compared to those listed. Yields of oil, polyphenol, hydrocarbon and protein are shown in Table 2. Seven species of the 110 yielded high quantities of oil (7.1-11.1% moisture-free plus ash-free sample weight basis) as well as moderate to substantial quantities of polyphenol. Yields are given on a moisture-free plus ash-free basis because plant samples have undetermined amounts of surface dirt. In order of decreasing oil yields these were *Juniperus scopulorum* from CO (11.1% oil + 17.1% polyphenol), *Pinus albicaulis* from CO (10.1% oil + 19.6% polyphenol), *Pinus flexilis* from CO (9.3% oil + 18.2% polyphenol), *Pinus mugo* from CO (8.4% oil + 14.7% polyphenol), *Liatris punctata* from ND (8.0% oil + 10.0% polyphenol), *Juniperus communis* from CO (7.8% oil + 19.0% polyphenol), and *Pinus aristata* from CO (7.1% oil + 19.7% polyphenol).

TABLE 1

General Information for Selected Plant Species Analyzed

Family species common name	Herbarium voucher number	Life cycle and habit	Height, m	Collection site	Primary geographic distribution in northern hemisphere
Aceraceae					
Acer ginnala Maxim. Amur maple	80415	deciduous shrub	to 6.2	Spruce Recreation Facility, Boulder, Colorado	Maine to Connecticut and western New York, zone 5
Caprifoliaceae					
Lonicera morrowii A. Gray Honeysuckle	80426	deciduous shrub	to 2.5	University of Colorado campus, Boulder	Maine to Michigan, south to Long Island, New Jersey and Pennsylvania, zone 4
Compositae					
Chrysothamnus graveolens (Nutt.) Greene Rabbit brush	80913	deciduous shrub	to 1.5	Northwest of Killdeer, North Dakota, on eroded buttes	North Dakota and Idaho, south to New Mexico and northern Arizona
Liatris punctata Hook. Narrow-leaved blazing star	80620	perennial herb	to 0.3	dry prairie, Mandan, North Dakota	Manitoba to Alberta, south to Iowa, Kansas, Texas and northern New Mexico
Lygodesmia juncea (Pursh) D. Don Skeleton weed	80617	perennial herb	to 0.12	dry sandy prairie, near Mandan, North Dakota	Wisconsin to Alberta, south to Missouri, Oklahoma, Texas and New Mexico
Cupressaceae					
Juniperus chinensis L. Pyramid chinese juniper	80421	evergreen tree	to 18.5	Roosevelt National Park, Boulder, Colorado	Zone 4
Juniperus communis L. Common juniper	80525	evergreen tree or shrub	to 10.8	Roosevelt National Park, Boulder, Colorado	Southern Maine to Manitoba, south to Maryland, Georgia, Indiana, Illinois, zone 3
Juniperus horizontalis Moench Creeping juniper	80927	evergreen shrub	to 0.3	prairie, sandy slope, southwest of Mandan, North Dakoa	Nova Scotia to Arkansas, south to New Jersey, Minnesota and Montana, zone 3
Juniperus scopulorum Sarg. Rocky mountain juniper	80928	evergreen tree	to 9.5	University of Colorado campus, Boulder	British Columbia, south to Arizona and Texas
Pinaceae					
Abies lasiocarpa (Hook.) Nutt. Alpine fir	80430	evergreen tree	to 30.5	Roosevelt National Park, Boulder, Colorado	Arkansas to New Mexico, zone 3
Picea engelmannii Parry ex Engelm. *Engelmann spruce*	80427	evergreen tree	to 45.7	Roosevelt National Park, Boulder, Colorado	British Columbia to New Mexico, zone 3
Picea pungens Engelm. Colorado blue spruce	80428	evergreen tree	to 30.5	University of Colorado campus, Boulder	Wyoming, Vermont, Colorado, New Mexico, zone 3
Pinus albicaulis Engelm. White-bark pine	80418	evergreen tree	to 27.4	Bridger National Forest, Sublette, Colorado	British Columbia to California (mountains), zone 4
Pinus aristata Engelm. Bristle-cone pine	80413	evergreen tree	to 12.2	Arapahoe National Forest, Clear Creek, Colorado	California to Colorado (mountains), zone 6
Pinus contorta Dougl. ex Loud Short pine	80524	evergreen tree or shrub	to 9.1	Roosevelt National Park, Boulder, Colorado	Arkansas to California, zone 7b
Pinus flexilis James Limber pine	80411	evergreen tree	to 18.3	Roosevelt National Park, Boulder, Colorado	Alberta south to California and Texas, zone 4
Pinus mugo Turra Mountain pine	80520	evergreen tree	to 9.1	University of Colorado campus, Boulder	Zone 3
Pinus ponderosa Dougl. ex P. Laws & C. Laws Western yellow pine	80412	evergreen tree	to 61.0	Genesee Park, Clear Creek, Colorado	British Columbia to Texas and Mexico, zone 6
Pseudotsugo menziesii (Mirb.) Franco Douglas fir	80419	evergreen tree	to 91.5	Clear Creek, Colorado	British Columbia to Texas and Mexico, zone 6
Rosaceae					
Sorbus sitchensis M. J. Roem. Mountain ash	80521	evergreen shrub	to 4.6	University of Colorado campus, Boulder	Arkansas to British Columbia and Idaho, zone 5
Rubiaceae					
Galium boreale L. Northern bedstraw	80714	perennial herb	to 0.9	Prairie, Mandan, North Dakota	Manitoba to Arkansas, south to West Virginia and New Mexico
Taxaceae					
Taxus canadensis Marsh. American yew	80522	evergreen shrub	to 1.8	University of Colorado campus, Boulder	Northern Florida to Virginia and Iowa, zone 3

TABLE 2

Chemical Analyses of Plant Samples

Species	Herbarium voucher number	Yield, %[a]				
		Oil	Polyphenol	Hydrocarbon	Protein	Total[b]
Abies lasiocarpa	80430	5.6	18.5	<0.1	6.1	30.2
Acer ginnala	80415	5.6	25.5	0.2	7.6	38.9
Chrysothamnus graveolens	80913	2.7	19.1	0.1	10.4	32.3
Galium boreale	80714	5.6	7.7	0.3	7.5	21.1
Juniperus chinensis	80421	4.7	15.8	0.2	6.4	27.1
J. communis	80525	7.8	19.0	<0.1	4.6	31.4
J. horizontalis	80927	5.8	9.8	0.2	7.7	23.3
J. scopulorum	80928	11.1	17.1	0.2	8.3	36.7
Liatris punctata	80620	8.0	10.0	0.1	7.5	25.6
Lonicera morrowii	80426	3.6	18.9	0.4	7.1	30.0
Lygodesmia juncea	80617	4.8	7.6	0.1	9.3	21.8
Picea engelmannii	80427	3.7	25.4	<0.1	5.5	34.6
P. pungens	80428	4.4	21.4	<0.1	8.3	34.1
Pinus albicaulis	80418	10.1	19.6	0.1	5.2	35.0
P. aristata	80413	7.1	19.7	0.1	4.7	31.6
P. contorta	80524	5.8	17.2	<0.1	4.8	27.8
P. flexilis	80411	9.3	18.2	0.3	4.2	32.0
P. mugo	80520	8.4	14.7	0.5	6.7	27.8
P. ponderosa	80412	6.6	22.1	0.1	5.9	34.0
Pseudotsugo menziesii	80419	5.4	24.7	<0.1	4.8	34.9
Sorbus sitchensis	80521	4.8	19.0	<0.1	6.3	30.1
Taxus canadensis	80522	3.2	19.9	0.1	8.9	32.1

[a]% is on a moisture- plus ash-free plant sample weight basis.
[b]Total % of oil + polyphenol + hydrocarbon + protein. Protein = % Kjeldahl nitrogen × 6.25.

Liatris punctata is a perennial herb. The other six species are evergreen trees or shrubs. Only 14 species of about 1100 previously analyzed at NRRC have yielded 8% or more oil. The two highest yields for the previously analyzed species were 11.2% for *Euphorbia dentata* Michx. and 9.9% for *Euphorbia lathyris* L. About 50 species of the 1100 have yielded 19% or more polyphenol but only 14 have yielded 25% or more. Six species of the 1100 have yielded 30% (*Rhus typhina* L.) to 36% (*Rhus copallina* L.) polyphenol.

Seven species listed in Table 2 gave 5.4-6.6% oil (*Pinus ponderosa*, 6.6%), and five of these gave 17.1-24.7% polyphenol (*Pseudotsugo menziesii*, 24.7%, and *Pinus ponderosa*, 22.1%). About 75 species of the 1100 have given at least 5% oil, and about 100 had at least 15% polyphenol. However, species yielding substantial amounts of both oil and polyphenol are atypical. Generally, such species have been trees or shrubs, particularly evergreens.

Another four species listed in Table 2 gave 4.4-4.8% oil plus 15.8-21.4% polyphenol. The remaining four gave low to very moderate yields of oil (2.7-3.7%) but gave substantial yields of polyphenol (18.9%-25.4%). Except for the first seven species discussed, the most noteworthy with respect to oil + polyphenol yields are *Acer ginnala* (5.6% + 25.5%), *Pinus ponderosa* (5.9% + 22.1%), *Pseudotsugo menziesii* (5.4% + 24.7%) and *Abies lasiocarpa* (5.6% + 18.5%).

Few species analyzed at this Center have contained 2% or more hydrocarbon. The two species in Table 2 that had 0.4% or more hydrocarbon were *Lonicera morrowii* (0.4%) and *Pinus mugo* (0.5%). The hydrocarbon fraction of *L. morrowii* contained rubber with a weight-average molecular weight (MW) of 64,100 and a molecular weight distribution (MWD) of 2.0, whereas hydrocarbon of *P. mugo* was waxy materials with an MW of less than 10,000. Hydrocarbon was not analyzed if the yield was less than 0.4%. The MW's of rubber-bearing species usually have been less than 250,000, rarely over 300,000 and never over 500,000, except for *Parthenium argentatum* A. Gray (1,280,000) and *Hevea brasiliensis* Mull. arg. (1,310,000) (16).

Two species unlisted of the 100 examined, which had rather low yields of oil and polyphenol, contained significant amounts of gutta. These were *Agropyron cristatum* (1.1% hydrocarbon) and *Agropyron smithii* (0.6% hydrocarbon) of the Gramineae. Of 85 grass species previously analyzed, seven have contained gutta with a maximum yield of 1.9% hydrocarbon for *Agropyron repens*.

Many species listed in Table 1 are referenced frequently in the literature for various reasons. However, they have received limited, if any, study with respect to chemical identification and characterization of specific constituents. Often any chemical study has involved a specific portion of the tree such as the leaves, bark, roots or fruit. Terpenes are the constituents more frequently studied of the *Pinus* and *Juniperus* species (*Chemical Abstracts*). Many evergreen trees such as *Pinus ponderosa, Pinus albicaulis, Pseudotsugo menziesii* and *Picea engelmannii* are valuable sources of commercial products such as lumber, turpentine, rosin and pulp.

Table 3 shows classes of oil constituents for species that yielded 5% or more oil. TLC indicated that all of

TABLE 3

Analyses of Plant Oils

Species	Herbarium voucher number	Oil yield %[a]	Classes of oil constituents[b]: Fatty acids fatty alcohols and sterols	Esters	Hydrocarbons	Saponified oil: Unsaponifiable matter %[c]	Fatty acids %[c]
Abies lasiocarpa	80430	5.6	yes	NG	t	39	38
Acer ginnala	80415	5.6	yes	TG	t	48	41
Galium boreale	80714	5.6	yes	NG	no	41	43
Juniperus communis	80525	7.8	yes	TG	t	35	39
J. horizontalis	80927	5.8	yes	TG, NG	t	51	43
J. scopulorum	80928	11.1	yes	NG, TG, TT	t	36	43
Liatris punctata	80620	8.0	yes	NG	t	40	38
Pinus albicaulis	80418	10.1	yes	TG	t	17	62
P. aristata	80413	7.1	yes	TG	t	15	63
P. contorta	80524	5.8	yes	TG	t	26	51
P. flexilis	80411	9.3	yes	TG	t	19	60
P. mugo	80520	8.4	yes	NG	no	50	36
P. ponderosa	80412	6.6	yes	TG	t	16	54
Pseudotsugo menziesii	80419	5.4	yes	NG	no	39	37

[a]See Table 2, footnote *a*.
[b]NG, nonglyceride; TG, triglyceride; TT, triterpenol; t, trace. Classes were indicated by thin layer chromatography.
[c]Percent of oil sample weight.

these oils contained sterols, fatty alcohols less polar than sterols (higher R_f), fatty acids and at least one type of ester. All oils of the *Pinus* and *Juniperus* species, except *P. mugo*, contained triglyceride (TG) esters. *P. mugo* oil contained nonglyceride (NG) esters. *J. horizontalis* contained NG as well as TG esters. *J. socpulorum* contained triterpenol (TT) esters as well as NG and TG esters. Oils of the other species listed in Table 3 contained NG esters. most oils contained small amounts of some type of hydrocarbon such as terpenes, waxes and/or low molecular weight polyisoprenes. These oil fractions as well as the polyphenol fractions need compositional characterization to assess their potential importance for industrual utilization. Species are now being selected from all species analyzed in this program for more detailed study of the solvent-extractable constituents.

Table 3 also gives yields of "fatty acids" (FA) and unsaponifiable matter (UM). The yields of FA represent total organic acids found after saponification of the oil, which are usually mostly free fatty acids. In general, the saponified plant oils have more UM than common seed oils. Of oils analyzed in this program, most have contained about 40-60% UM. All oils of the *Pinus* species, which apparently contained essentially all TG esters, had low amounts of UM (4 oils with 15-19% and one with 26%). Oils of all other species listed contained 35-51% UM (36-43% FA). The combined amounts of UM and FA frequently are in the range of 70-90% of the oil sample. The range is 70-94% for the 14 species listed and 74-79% for 10 of them. Some volatile materials are lost during oven drying of the UM and FA fractions. Also, some materials such as glycerol, low molecular weight alcohols and sugars that are obtained in final partitioning of the saponified oils were not quantitatively analyzed.

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