diminished by tightly packing imbibed grain in a sealed container. Future research will need to determine whether HCl or H_2O treatments are applicable to other high tannin varieties. Nutritional studies will also have to be conducted to determine whether further improvements in protein digestibility and PER may be possible with prolonged storage (10–20 days) of water- or acid-treated sorghum.

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Oleoresins of Pinyons

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Monoterpenoid hydrocarbons from wood and gum turpentines of 11 pinyon species and varieties are composed of α-pinene, camphene, β-pinene, 3-carene, sabinene, myrcene, limonene, β-phellandrene, γ-terpinene, terpinolene, and *p*-cymene with occasional occurrence of *cis*-ocimene, tricyclene, α-phellandrene, thujene, and α-terpinene, generally in trace amounts. Higher boiling constitutents include at least 27 sesquiterpenoid hydrocarbons and 14 oxygenated monoterpenoids and volatile nonterpenoids. Pimaric acids, mainly Δ-8(9)-isopimaric acid, account for about 70% of the resin acids, with the rest composed mainly of abietic-type acids. Paraquat treatment of the singleleaf pinyon produces negligible resinification within 1 year.

Pinyons are pines of semiarid regions of the southwestern United States and central and north central Mexico (Critchfield and Little, 1966). They are relatively small in size compared to western pines such as ponderosa pine, lodgepole pine, or western white pine, or to southeastern pines such as loblolly pine or slash pine, and often grow in association with other small size tree species, such as junipers. They are slow-growing species (Barger and Ffolliott, 1972), commonly bushy in appearance with branches down to the ground, with the result that about half of the weight of a tree represents leaves and branches and with wood including a large number of knots and other defects. Pinyons comprise 11 species and varieties, with *P. cembroides*, *P. edulis*, and *P. monophylla* responsible for most pinyon stands in existence and with the rest covering either considerably more restricted ranges or known from one or a few localities only (Critchfield and Little, 1966).

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Pinyon forests comprise about 61 million acres in the United States (Barger and Ffolliott, 1972) and about 45 million acres in Mexico. Forests of Arizona and New Mexico contain about 393 ft^3 of wood per acre in the north of the states and only 23 ft^3 per acre for the woodland zone of the south. These figures change to 744 and 595 ft^3 per acre, respectively, if one includes junipers which commonly occur together with pinyons (Howell, 1940). While this is well below the volume of softwood available on an acre of commercial timberland in the Pacific U.S. region (over 3000 ft^3/acre), it compares favorably with that of southeastern states (from 266 ft^3/acre for Virginia to 513 ft^3/acre for South Carolina) (McGuire, 1973). At the same time pinyons and junipers are lagging well behind the southern pines in terms of annual growth—6.0 and 3.6 ft^3/acre for northern Arizona and New Mexico, respectively, vs. about 28 ft^3/acre for southeastern U.S.

Commercial utilization of pinyons is not extensive. The defective nature of wood limits its use as lumber and results in a low quality veneer for plywood. The pulp of *P. edulis* and *P. monophylla* is short fibered, which is undesirable and limits its use in pulp and paper (Barger and

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Ffolliott, 1972; Brink, 1978). Furthermore, pinyon forests are generally located in regions where unavailability of water in sufficient amounts for chemical pulping would involve costs of transporting the chips elsewhere. The possibility of using pinyon wood in the production of particle boards may have some potential, although no data are available. Pinyons were found to be inferior producers of gum oleoresin compared with southern pines (Betts, 1912; Deaver and Haskell, 1955). Presently the pinyons are used for railroad ties and mine timbers, firewood, Christmas trees, and as the source of pinyon nuts. The latter has been estimated to be worth one to several million dollars a year (Fowells, 1965).

To these largely commercial aspects must be added, in all fairness, the value of pinyons as ground cover, in erosion control, for wildlife food and habitat, in recreation, etc. These topics, while real, are outside of the scope of this paper, however, and are not going to be discussed.

In 1972 on the basis of a comprehensive review of the subject, Barger and Ffolliott concluded that utilization of woodlands of the region including pinyons is unattractive to private industry due to the low volume per acre, high harvesting costs, and low quality of potential products. It cannot be excluded that this situation could change in the future in connection with the continuously mounting energy shortage, and increasing demand and costs of wood and wood-related products, as well as with the newer methods of tree utilization, such as use of Paraquat and related herbicides for increasing the resin content of wood, nonconventional methods of wood bonding, whole tree utilization methods, and the like.

In this paper we are reporting on the composition of Naval Stores from pinyon wood and discussing the data from the point of view of the potential utilization of these products, a topic which certainly must be considered in any future planning of commercial use of these currently underutilized species.

RESULTS

Wood Turpentines. Commerical wood turpentine is produced by solvent extraction of oleoresin from stumps remaining from logging operations and distillation of the extract. It is closely related to sulfate turpentine, obtained as a byproduct of alkaline pulping.

The composition of the lower boiling fraction of wood turpentines (essentially monoterpenoid hydrocarbons) has been determined in this laboratory for wood of all known pinyon species and varieties by independent analysis of about 1000 wood samples, each from an individual tree, collected in about 100 different locations. The means for 10 trees are summarized in Table I for representative stands of each species. The variability between trees within a locality was minor in comparison with the trends noted. Where tested, variation within an individual tree was negligible.

The majority of pinyons, including the three most important ones, produces largely α-pinene (Figure 1). α-Pinene is also the main component of most commercial turpentines and represents the starting synthetic material for many important pharmaceuticals (insecticides, perfumes, vitamins), pine oil, and other synthetics (Zinkel, 1975; Derfer, 1977; Ansari, 1970). *P. pinceana* and *P. maximartinezii* produce largely limonene. This terpenoid is also the main constitutent of the turpentine from *P. pinea* of southwestern Europe and is convertible to a variety of useful products. The synthetically important transformations of limonene have been reviewed by Verghese (1968, 1969). *P. nelsonii* produces, besides ubiquitous α-pinene, β-pinene in larger amounts; the latter

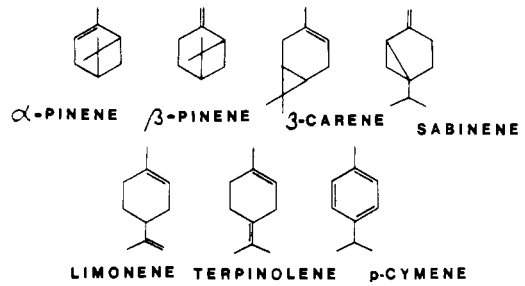

Figure 1. Structures of some monoterpenes from pinyons.

is a particularly desired component and represents a starting material in many synthetic applications (Ansari, 1970; Zinkel, 1975; Derfer, 1977). Unfortunately all three pine species are rather rare, which limits them for commercial considerations. The rare *P. culminicola* of Mexico and more common *P. cembroides* var. *bicolor*, primarily of southern Arizona and New Mexico and northernmost Mexico, are particularly interesting with turpentines composed of α-pinene, sabinene, terpinolene, and *p*-cymene in larger amounts. In one tree of var. *bicolor*, *p*-cymene was the most abundant constitutent (44.9%). These two pines represent the only pines known so far producing *p*-cymene in amounts as large as 10% or more. Unfortunately *p*-cymene is a generally available compound, and this additional source is of little interest commercially. At the same time both sabinene and terpinolene represent less common chemicals and offer a definite chemical synthetic opportunity due to their unique chemical structures and reactivity. Sabinene transforms easily into α-thujene (Acharya et al., 1969) or a mixture of α- and γ-terpinenes, terpinolene, and terpinen-4-ol (Cooper et al., 1973; Wrolstad and Jennings, 1965). The chemistry of terpinen-4-ol, terpinolene, and α-terpinene has been recently reviewed (Verghese, 1966, 1967, 1972).

The higher-boiling constituents are present in pinyon wood in relatively high amounts (Table I), about equaling those of monoterpenoid hydrocarbons. They are composed in the main of sesquiterpenoid hydrocarbons, the oxygenated monoterpenoids being only secondary in importance. The constituents are likely to be at least qualitatively the same as those of gum turpentines. The large content of high boiling compounds in pinyons contrasts strongly with that of southern pines which generally include only a low proportion of higher boiling components.

Gum Turpentines. Gum turpentines are produced by distillation of gum oleoresin, collected as exudate after wounding the pine trees.

The gum oleoresin potential of the *P. edulis* has been investigated by Betts (1912) in Colorado. The average yield amounted to about two-thirds of that secured from ponderosa pine in Arizona and to a little over half the average yield of longleaf pine in Florida when calculated for the same flow period. Additional experiments were performed by Deaver and Haskell in northeastern Arizona (1955). Twenty-seven percent of trees were nonproductive; the remaining 73% of trees produced 0.65 kg of oleoresin each in the 4-month period between June and September. For comparison, *P. elliottii* (slash pine) in southeastern United States produces between 1.5 and 9.2 kg per 8–9 month period, depending upon site, tree diameter, and crown ratio (USDA, 1935; Harrington, 1969; Bengtson and Schopmeyer, 1959).

The turpentine content of gum oleoresin from pinyons is generally in line with that of other pines (Table II). α-Pinene is present as (+) enantiomer in all pinyons investigated except for *P. nelsonii*; the rotations of other

Table I. Composition (%) of Turpentines from Wood of Pinyon Species[a]

no.	botanical name	trivial name	state	compounds[b] A	B	C	D	E	F	G	H	I	J	K	% of monoterpenoid hydrocarbons in wood	% of higher boilers in wood
1	*P. cembroides* Zucc. var. *cembroides*	Mexican pinyon	Zacatecas	88.4	1.0	1.6	2.0	1.6	0.5	4.1	0.2	0.1	0.3	0.3	0.07	0.12
2	*P. cembroides* Zucc. var. *remota*, Little	Texas pinyon	TX	72.1	3.1	5.0	0.7	0.3	0.6	9.1	1.4	0.1	0.2	1.0	0.06	0.09
3	*P. cembroides* Zucc. var. *bicolor*, Little		AZ	37.4	2.0	3.2	0.2	23.3	2.2	3.4	2.5	2.4	6.1	14.3	0.10	0.06
4	*P. culminicola* Anderson & Beaman	Potosi pinyon	Nuevo Leon	30.9	0.2	1.4	6.4	4.1	4.3	14.4	2.3	0.5	13.7	8.8	0.10	0.07
5	*P. pinceana* Gord.	Pince pinyon	Querretaro	5.0	1.3	0.8	0.3	0.1	1.8	90.2	0.1	Tr	0.2	0.1	0.20	0.15
6	*P. maximartinezii* Rzedowski	Martinez pinyon	Zacatecas	7.3	0.5	0.6	0.1	Tr	2.7	88.6	Tr		0.2	0.1	0.21	0.12
7	*P. nelsonii* Shaw	Nelson pinyon	Tamaulipas	57.8	0.9	32.8	0.2	Tr	2.2	5.5	0.3	Tr	0.2	0.1	0.03	0.02
8	*P. edulis* var. *edulis* Engelm.	Colorado pinyon	NM	81.5	1.1	0.9	10.0	Tr	1.8	2.4	0.2	0.2	0.7	0.8	0.06	0.03
9	*P. edulis* var. *fallax* (Engelm.) Little		NV	91.7	1.5	0.6	0.2	Tr	0.8	3.2	0.2	0.1	0.9	0.4	0.06	0.15
10	*P. monophylla* Torr & Frém.	singleleaf pinyon	NV	86.4	1.6	0.7	3.4	0.2	1.3	4.2	0.2	0.1	0.5	0.9	0.07	0.06
11	*P. quadrifolia* Parl.	Parry pinyon	Baja Calif.	93.0	1.4	1.0	Tr		1.4	1.6	Tr	Tr	0.3	0.1	0.11	0.12

[a] Additionally: *cis*-ocimene, 1.2% in no. 11; 0.3% in no. 8, 10; 0.4% in no. 9; Tr in no. 2, 6, 7; 11.5% in no. 4; 0.7% in no. 3. Tricylene, Tr in no. 1, 5, 6, 11; 0.2% in no. 2, 9, 10; 0.1% in no. 3, 8. α-Phellandrene, Tr in no. 5; 0.4% in no. 4. α-Thujene, 1.1% in no. 4; 2.0% in no. 3. α-Terpinene, 0.1% in no. 3. [b] Compounds: A, α-pinene; B, camphene; C, β-pinene; D, 3-carene; E, sabinene; F, myrcene; G, limonene; H, β-phellandrene; I, γ-terpinene; J, terpinolene; K, *p*-cymene.

Table II. Composition (%) of Turpentines from Gum Oleoresin of Pinyon Species[a]

no.	Latin name	ref	collection locality	compounds A	B	C	D	E	F	G	H	I	% of higher boilers[a]	% of volatiles in oleoresin
1	*P. cembroides*	Mirov (1951)	Coahuila	+96.0						−4.0			2.0[b]	26.2
5	*P. pinceana*	Mirov (1951)	Coahuila	+5.9						−94.1			5.9[b]	17.7
7	*P. nelsonii*	Mirov et al. (1962)	Tamaulipas	−53.7	0.3	−44.9				1.1			3.9[c]	27.8
8	*P. edulis* var. *edulis*	Snajberk and Zavarin (1975); Mirov and Iloff (1956)	NM and UT	+74.6	0.4	0.4	+14.5	3.5	5.4	Tr	1.0	0.2	34.9[b]	27.1
10	*P. monophylla*	Zavarin et al. (1971)	CA	+74.5	1.0	0.5	+6.0	3.0	3.0	−8.0	1.5	2.5	23.0[b]	24.5
11	*P. quadrifolia*	Iloff and Mirov (1954)	CA	+94.3								5.7	28.0[b,d]	23.5

[a] Content on monoterpenoid hydrocarbons and higher boiling materials was calculated for monoterpenoid hydrocarbons = 100%; +/− indicate the most abundant enantiomer. Compounds: A, α-pinene; B, camphene; C, β-pinene; D, 3-carene; E, sabinene; F, myrcene; G, limonene; H, terpinolene; I, *cis*-ocimene. [b] Mainly sesquiterpenoid hydrocarbons. In no. 8 and 10 ocimene was erroneously originally reported as trans. [c] Includes traces of heptane; higher boilers contained unidentified oxygenated materials. [d] On the basis of the reported 235 nm maximum in UV (EtOH) ocimene should have been cis (Hellyear and Lassak, 1968).

Table III. Composition (%) of the Higher-Boiling Gum Turpentine Constituents from *P. edulis* and *P. monophylla*

composition	*P. edulis*[c]	*P. monophylla*[c]
sesquiterpenoid hydrocarbons		
total[a]	80.5	89.3
β-farnesene	1.3	6.8
germacrene D	29.2	8.7
α-muurolene	0.5	6.0
γ-muurolene	2.2	2.9
γ-cadinene	3.7	6.1
δ-cadinene	1.4	3.0
γ-amorphene	8.1	10.4
α-guaiene		1.3
δ-guaiene	Tr	0.6
α-cubebene	0.6	1.3
α-copaene	6.6	10.4
β-copaene	1.9	
β-ylangene	0.9	1.6
sativene	Tr	1.1
cyclosativene	3.2	4.5
β-bourbonene	Tr	0.7
α-humulene	Tr	0.7
γ-humulene	0.2	2.1
caryophyllene	2.0	2.6
longifolene	14.8	16.4
α-longipinene	1.0	0.9
longicyclene	0.6	Tr
others		
total[a]	19.5	10.7
linalool	Tr	0.7
bornyl acetate	1.2	2.2
borneol	Tr	0.6
verbenone	0.9	0.8
ethyl caprylate	14.8	2.6
higher boilers in turpentine[b]	34.9	23.0
higher boilers in oleoresin (%)	7.08	5.15

[a] In traces (<0.5%): β-cadinene, ε-cadinene, α-amorphene, calamenene, sibirene, camphor, terpinen-4-ol, citronellyl acetate, neral, α-terpineol, citronellol, nerol, geraniol, and methyl chavicol. Composition expressed in percent of their total. [b] In percent of monoterpenoid hydrocarbons = 100%. [c] Recalcuated from Snajberk and Zavarin (1975) and Zavarin et al. (1971).

monoterpenoids correspond to those generally encountered in pine turpentines—(−)-limonene, and (−)-β-pinene. The proportions of the higher boiling sesquiterpenoids and oxygenated monoterpenoids are somewhat lower in gum turpentines than in wood turpentines. As in wood turpentines the bulk of the higher boiling materials is made up of sesquiterpenoid hydrocarbons.

Gum turpentines of *P. edulis* and *P. monophylla* were investigated recently by a combination of GLC and spectroscopic methods (Zavarin et al., 1971; Snajberk and Zavarin, 1975) (Tables II and III). Compounds other than terpenoids were rather low, with exception of ethyl caprylate in *P. edulis* (Mirov and Iloff, 1956). This compound is a rare constituent of pine turpentines and is responsible for the characteristically pleasant fragrance of the *P. edulis* oleoresin. Unfortunately it is of little value commercially, being readily obtainable through synthesis. Both oxygenated monoterpenoids and sesquiterpenoids were composed of a large variety of compounds, most of them in small or trace amounts. α-Muurolene, γ-muurolene, γ-cadinene, α-copaene, γ-amorphene, caryophyllene, longifolene, and germacrene D (Figure 2) were responsible for about 67% of the higher boiling materials in *P. edulis* and for 64% in *P. monophylla*. None of these compounds are commercially available at the present time and their synthetic potential is unknown. The presence of 7.6% of germacrene D in gum turpentine of *P. edulis* is interesting due to unusual structure, and ease of purification through

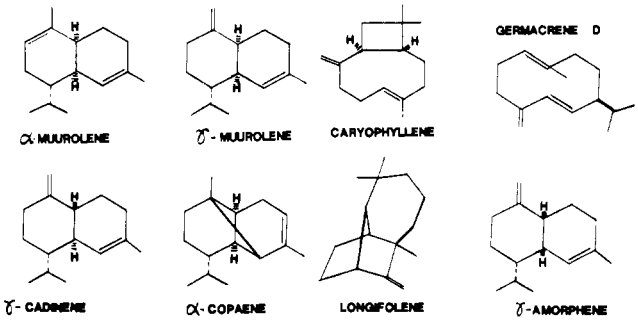

Figure 2. Structures of some sesquiterpenes from pinyons.

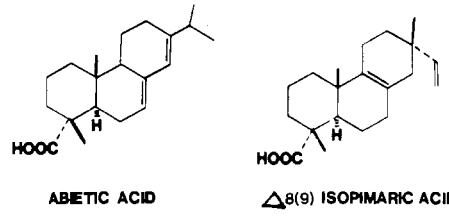

Figure 3. Structures of some resin acids from pinyons.

π-complexing with silver ion (Snajberk and Zavarin, 1975). The information on composition of the higher-boiling materials from gum turpentines of the remaining pinyons is scanty (Mirov, 1961). The compounds identified included (+)-longifolene and "cadinenes" in addition to several unknowns.

Wood and Gum Rosins. The information on composition of wood and gum rosins from pinyons is rather incomplete (Table IV). What is available indicates, however, that it differs appreciably from the composition of conventional rosins from southern pines. Roughly 70% of the rosin represents pimaric rather than abietic acids, with the rare Δ-8(9)-isopimaric acid (Figure 3) strongly predominating. According to data given by Joye and Lawrence (1967), the proportion of pimaric acids in rosin from 13 important rosin producing species does not even approach these figures. Pimaric acids are generally more stable than abietic acids because of the absence of conjugated double bonds and impossibility to easily isomerize to such due to separation of the two isolated double bonds by a quaternary carbon. The high percentage of pimaric acids is most likely responsible for superior performance of the pinyon gum rosin in varnishes (Colton, 1948). The use of pinyon gum oleoresin as varnish has been known for a long time to Hopi Indians.

Response to Paraquat. As well known (Lightwood Research Coordinating Council, 1975–1977), introduction of solutions of Paraquat and related compounds into wood of pine trees produces in 1–3 years intensive resinification of wood in the area above the wound. No information on the response of pinyons to such a treatment is currently available, although so far all pine species showed the resinification effect. Treatment of *P. monophylla* with 5 mL of 2, 4, or 8% of aqueous Paraquat solutions produced an appreciable visual effect within 1 year with branches above the wound dying off and copious exudations of oleoresin appearing on the bark. At the same time no definite increase in benzene-soluble materials could be noted in wood 45 or 90 cm above the wound (average 1.9% vs. 2.0% for unaffected wood). Under the same conditions southern pines have been demonstrated to increase their benzene extracts many fold (Lightwood Research Coordinating Council, 1975–1977). It is possible that the cold weather conditions prevailing most of the year in the experimental location are responsible for the lack of response observed

Table IV. Composition of the Resin Acids from Oleoresins of Pinyons[a] (in Percent of Their Total)

species:	*P. edulis*			*P. monophylla*		*P. quadrifolia*	
data source:	Joye and Lawrence (1967); Anderson et al. (1969)			Anderson et al. (1970)		Anderson et al. (1970)	
source of resin acids:	gum rosin	sapwood rosin	heartwood rosin	sapwood rosin	heartwood rosin	sapwood rosin	heartwood rosin
locality:	Ng[b]	Ng	Ng	San Bernardino Co., CA		Riverside Co., CA	
abietic type	33.9	20.0	34.6	22.7	33.0	28.0	20.0
abietic acid	21.0	16.4	2.2	6.8	14.0	14.7	7.0
neoabietic acid	5.4		8.7	4.5	13.0	4.0	7.0
levopimaric acid and palustric acid	5.3	3.6	10.7	11.4	6.0	9.3	6.0
dehydroabietic acid	2.2		13.0	Tr	Tr	Tr	Tr
pimaric type	64.2	78.2	61.1	77.3	65.0	68.0	75.0
Δ-8(9)-isopimaric acid	55.0	44.6	44.6	59.1	53.0	52.0	61.0
isopimaric acid	5.7	9.1	9.8	9.1	6.0	10.7	10.0
sandaracopimaric acid	3.5	5.5	6.5	9.1	6.0	5.3	4.0
pimaric acid		Tr		Tr	Tr	Tr	Tr
labdane type	1.3						
communic acid	1.3						
unidentified		1.8	4.3	Tr	2.0	4.0	5.0
% ether solubles in wood		3.2	4.6	2.0	9.4	1.8	5.1
% resin acids in wood		1.2	2.4	0.9	4.6	0.7	3.5

[a] Free fatty acids identified in wood included mainly oleic and linoleic acids, with caprylic, palmitic, arachidic, and linolenic acids as trace or secondary constituents. Free fatty acids were present mainly in sapwood, 0.1-0.9%, with little in heartwood (0.2%-Tr). Unfortunately fatty acid glycerides were not analyzed; thus it is impossible to estimate the total amount and composition of fatty acids. [b] Ng, not given.

and that more time must be allowed for resinification to take place.

EXPERIMENTAL SECTION

Twelve mature trees of *P. monophylla*, 2.5–6 m high, were treated with 5 mL of 2, 4 or 8% aqueous solutions (four trees per solution) of Paraquat L (1,1′-dimethyl-4,4′-bipyridinium dichloride) by the axe frill method about 30 cm above ground on Sept 15, 1977, in Toiyabe National Forest, Douglas Co., NV, off Highway 22, near Rickey mine at 2200 m elevation. The site was visited again on Sept 25, 1978. Oleoresin exudations were externally visible above the wounds on stem and branches, which were all alive with 2% treatments, and some dead and some alive with 4 and 8% treatments. The stems were cored at 45 and 90 cm above the wound. Reference samples were taken at the same height of the tree, but opposite to the wounded site. In addition, two trees not treated with Paraquat were cored as references.

For investigation of wood turpentines of the various pinyon species, ten healthy and mature trees per location were cored about 120 cm above the ground. In all cases increment wood cores (4 mm diameter, 12 cm long) were obtained, wrapped in aluminum plus polyethylene foil, and kept at −15 °C prior to analysis.

For GLC analysis of monoterpenoid hydrocarbons, the core was sliced into thin cross sections, weighed, and digested overnight covered with 2 mL of pentane containing 0.01% of nonane as internal standard. The pentane extract was reduced in volume by flash evaporation (Zavarin et al., 1979) and 1 μL of the resulting liquid was injected. Individual components were identified by relative retention times, using authentic internal standards. No isolation was deemed necessary as monoterpenoid hydrocarbons of wood from genus *Pinus* represent a well-known group of compounds, limited in number and resolvable by GLC. Column: aluminum, 3.2 mm × 3.0 m, 10% β,β-oxydipropionitrile on acid-treated, silanized Chromosorb P, 100/120 mesh, N_2 flow 10 mL/min. Temperature: injection port, 90 °C; column, 55 °C; detector, 55 °C. Detector: flame ionization. Quantitation: Varian CDS 101 Data System, data normalized to 100%.

For determination of benzene solubles, about 1 g of core slices was covered with measured 2.0 mL of benzene and digested overnight, and a measured aliquot of the extract was evaporated to dryness under high vacuum toward the end. The weight of the extract was expressed in percent of the 105 °C dried core slices.

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Chemical Modification of Carboxyl Groups in Porcine Pepsin

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Modification of up to 11 carboxyl groups in porcine pepsin with 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide and glycine methyl ester caused changes in activities, specificity, and physicochemical properties of the enzyme. The milk clotting activity was markedly decreased to 10%, while the proteolytic activity was not affected. The decrease in the peptidase activity was about 50%. The charge density of pepsin decreased upon modification, as shown in a decrease of relative electrophoretic mobility and in a shift of pH optimum from 2 to 3.5. Kinetic studies showed that K_m was increased, while k_{cat} was not significantly affected. The presence of dipeptide substrates interfered with the modification. The modified pepsin remained reactive to two site-specific pepsin inhibitors. These effects of carboxyl modification were not unique to pepsin; modification of carboxyl groups caused similar changes in the activities and properties of pepsinogen and chymosin. The stability of the modified pepsin near neutral pH was considerably improved, suggesting that the modified enzyme may be a more suitable rennet substitute than native pepsin in cheese-making.

Porcine pepsin (EC 3.4.23.1) has been used as a calf rennet substitute in cheese making (Bottazzi et al., 1976; Carbone and Emaldi, 1976; Phelan, 1973), but the use of pepsin alone is considered unsuitable (Green, 1972; Sardinas, 1972). The objective of this study was to change the activities, specificity, and physicochemical properties of pepsin by chemical modification, hoping that these alterations could enhance the potential utilization of pepsin in the manufacture of cheese. Carboxyl modification with water-soluble carbodiimides (Hoare and Koshland, 1967) has been found to change the properties of some enzymes (Matyash et al., 1973; Swaisgood and Natake, 1973). In the present study, the carboxyl groups in pepsin were modified and the changes in enzymatic activities, kinetics, and some physicochemical properties were investigated. The role played by the nonessential carboxyl groups of pepsin in its function was also assessed.

EXPERIMENTAL SECTION

Materials. Porcine pepsin (2× crystallized), pepsinogen, and chymosin were purchased from Sigma Chemical Co., St. Louis, MO. Pepsin was homogeneous as indicated by gel electrophoresis and DEAE-cellulose column chromatography at pH 4.2 and was used without further purification. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), *N*-acetyl-L-(phenylalanyl)-L-diiodotyrosine (APDT), *N*-acetyl-D-(phenylalanyl)-L-tyrosine, *N*-(carbobenzoxy)-L-glutamyl-L-tyrosine, and the methyl esters of arginine, leucine, lysine, tyrosine, and tryptophan were also products of Sigma Chemical Co. Glycine methyl ester was purchased from Aldrich Chemical Co., Montreal, P.Q. Two pepsin inhibitors, 1,2-epoxy-3-(*p*-nitrophenoxy)propane and bromophenacyl bromide, were purchased from Eastman Kodak Co., Rochester, NY.

Modification of Carboxyl Groups. The carboxyl groups in pepsin were modified by the carbodiimide-promoted amide formation. The method employed was essentially that of Hoare and Koshland (1967) except that dissociating agents were omitted and sufficiently lower concentrations of carbodiimide and nucleophile were used to achieve limited modification. The enzyme (10 mg/mL) and nucleophile were dissolved in distilled water, and the pH of the solution was adjusted to 5.5 with 0.1 N NaOH. The carbodiimide EDC was added as a solid to obtain the desired concentration. The reaction mixture was stirred

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