length. A decline in the amount down to about 880 mg/100 g was noted with further growth in pods until maturity. The vicine content of cotyledons also followed a similar pattern but the peak concentration could not be detected because the seed coats could not be removed from the very young seeds. The seed coat vicine content also changed with growth and its value, in all cases, was below that of the cotyledons and of the whole seeds.

It needs to be emphasized that, in the cultivar examined, the concentration of vicine was the highest when the pod length was about half of its mature length. This finding is not in agreement with that of Brown and Roberts (1972), who did not find any vicine in seeds within pods of less than 10 cm length. However, it is in accordance with the data of Jamalian et al. (1977b), indicating a high degree of toxicity associated with the immature broad bean seeds of two other broad bean cultivars (Rud Pish and Hendu Kola). Nevertheless, the discrepancy between our data and those of Brown and Roberts (1972) might be due to differences in cultivars, methods of extraction, etc., and should be further studied.

Determination of simple correlation coefficients and linear regression coefficients was based on all possible data pairs as shown in Figure 2. It can be noted that the correlation coefficients were highly significant (1% probability level) for the relationships between the vicine concentration in different parts of the seeds and the intact seed. This coefficient was especially large and the slope of the regression line was steeper for the cotyledon vs. the whole seed because cotyledons constitute the major portion of the seed. Nonlinear regressions were also tried for the data; however, linear relationships fitted the data best.

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Composition of Jojoba Seeds and Foliage

High-performance liquid chromatography and thin-layer chromatography methods were developed to assay two major toxicants in jojoba plant tissues. Simmondsin, the most prevalent toxicant, is present in seeds at 2.3% levels and is also found in hulls, leaves, twigs, core wood, and male inflorescence. Simmondsin 2′-ferulate, the second most prevalent toxicant, occurs in seeds but was not found in the other plant tissues investigated. Oil, protein, carbohydrate, and amino acid levels are also reported for the seeds.

Jojoba [*Simmondsia chinensis* (Link) Schneider] is a dioecious desert shrub that grows naturally on arid lands in Arizona, California, and Mexico (National Academy of Sciences, 1975, 1977). Jojoba seeds vary in size from that of a coffee bean to a large peanut. A single mature shrub may produce as much as 5–10 lbs or more of seeds per year. The seeds contain about 50% of a colorless, odorless oil, that is structurally similar to sperm whale oil. The oil is unique in that it is essentially a mixture of monoesters containing monounsaturated carboxylic acids and alcohols with 20 and 22 carbon atom lengths. There is growing interest in jojoba oil in its own right, and as a replacement for sperm oil, a valuable commodity no longer available in the United States.

After removal of the oil from the seeds, the remaining meal is high in protein and is a potential animal feed ingredient. Although a few wild rodents, deer and swine eat the seeds (Sherbrooke and Haase, 1974), laboratory mice, rats, and poultry do not do well on the meal (Booth et al., 1974; Weber and Reid, 1977). The presence of several 2-cyanomethylenecyclohexyl glycosides (Elliger et al., 1973, 1974a,b), at least one of which has demonstrated toxicity, render the meal unsuitable as a livestock feed. The goal of detoxifying jojoba seed meal as a by-product for use in livestock feed has provided the impetus for developing assay methods for the toxicants and for determining the composition of the seeds and foliage.

Thin-layer chromatography and high-performance liquid chromatography methods were developed for assaying the two major toxicants, simmondsin and simmondsin 2′-ferulate. An assay of these two toxicants and the protein, amino acid, carbohydrate, fiber, and oil contents of seeds from two locations are reported. Assays for toxicants in leaves and twigs are included because these parts of the plant have provided browse for cattle. An assay of the hulls is reported because in commercial processes used to express the oil, the seeds contain as much as 10% or more hulls to facilitate pressing. Composition of the hulls is significant when the resulting meal is used as an animal feed.

EXPERIMENTAL SECTION

Materials. Seeds were obtained from the 1976 harvest of the Southern California Jojoba Project (SCJP 977) and

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the San Carlos Apache Jojoba Development Project (Apache 377). The foliage was supplied by Professor Richard W. Rice, Department of Animal Science and by Leslie Rawles, Office of Arid Lands Studies, University of Arizona. The branch from which the core wood was obtained was provided by Professor D. M. Yermanos, University of California, Riverside.

Test Materials. The dehulled seeds were ground in a laboratory mill until 75% or more of the material could be brushed through a 35-mesh screen. The resulting raw meal was deoiled by three successive extractions with hexane at ambient temperature. The deoiled meal was desolventized in air, then dried to constant weight in an oven at 65 °C prior to analysis. The dry foliage was separated into male inflorescence, leaves, and twigs, and each plant part was then similarly ground to a fine meal. Seed hulls were similarly ground prior to extraction.

Reference Standards. Hexane deoiled jojoba seed meal was extracted twice with acetone using a solvent–meal ratio of 2:1 (v/w) and refluxing for 4 h. After evaporation of the acetone, about 6.2 g of mixed toxicants are obtained as a tan, amorphous powder from 100 g of meal. These mixed toxicants were dissolved in excess acetone and washed through a short column containing Merck silica gel 60 (230–400 mesh), and the eluent was concentrated to a smaller volume. This acetone solution was allowed to evaporate slowly in a beaker and a product rich in simmondsin crystallized. Repeated recrystallization of the precipitate in this manner provided simmondsin hydrate, mp 95–100 °C (Elliger et al. 1973). This product had an identical R_f value when compared on TLC with an authentic sample provided by Dr. Anthony C. Waiss, Western Regional Research Laboratory, USDA, Albany, Calif. Its UV absorption was also similar to literature values.

Simmondsin 2′-ferulate is much more soluble in acetone than simmondsin and is obtained from the supernate. After removing the acetone, the residue was dissolved in excess ethyl acetate. The ethyl acetate was allowed to evaporate in a beaker, resulting in the precipitation of a product that is principally simmondsin. Much of the simmondsin 2′-ferulate remained in the ethyl acetate, and it was obtained as a gummy mass on evaporation of the remaining solvent. This material was precipitated as light-yellow amorphous product by dissolving in acetonitrile and adding the solution slowly to stirred isopropyl ether. Its absorption spectrum in ethanol had λ max 218 nm, log ε 4.32, and λ max 327 nm, log ε 4.20, which was similar to reported values (Elliger et al. 1974b). An elemental analysis indicated that the product may be partially hydrated. Anal. Calcd for $C_{26}H_{33}NO_{12}$: C, 56.62; H, 6.03; N, 2.53. Found: C, 55.95; H, 6.45; N, 2.48.

Ethanol solutions of pure simmondsin and simmondsin 2′-ferulate are stable, with no decomposition noticeable after 8 months.

High-Performance Liquid Chromatography. Samples for LC analysis were prepared by extracting 10 g of finely ground material with acetone for 6 h in a Soxhlet extractor. Plant parts other than seeds were extracted with hexane to remove oil prior to extraction of the toxicants with acetone. The acetone was removed from the extract under vacuum and the residue was brought to a volume of 10 mL in ethanol. A 1-mL aliquot was diluted with 3 mL of ethyl acetate. This solution was then passed through a column containing 0.5 g of silica gel 60 (230–400 mesh); the product was washed from the column with 10 mL of ethyl acetate–ethanol (7:3). The eluate was evaporated to dryness, the residue taken up in methanol, filtered, and brought to a known volume, usually 10 mL.

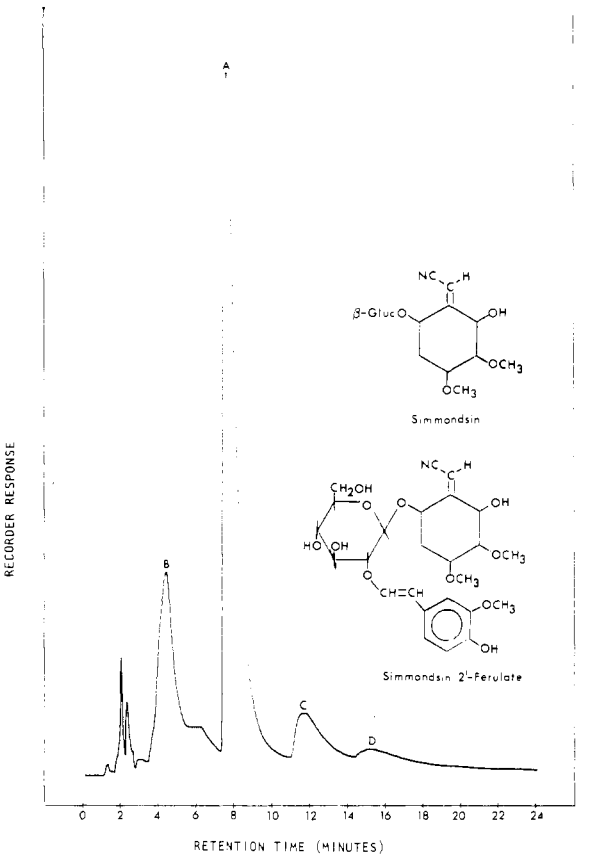

Figure 1. Typical jojoba seed meal high-performance liquid chromatogram: A, simmondsin; B, simmondsin 2′-ferulate; C and D, probably two minor toxicants (Elliger et al., 1974b); structure of simmondsin (Elliger et al., 1973 and 1974a).

This technique removed most of the material which appeared at the origin on TLC monitor plates.

LC analysis of this column-purified sample was carried out using an Altex Model 110 solvent metering pump and an Altex/Hitachi Model 155-30 variable wavelength UV-VIS detector. The main column 3.2 × 250 mm was packed with Lichrosorb Si 60, 5 μm, and protected by a precolumn 3.2 × 40 mm of Porasil A, 37–75 μm. Both simmondsin and simmondsin 2′-ferulate have strong absorption maxima at 218 nm, which facilitates quantitation, done by area measurement, even at low concentration levels. At 220 nm in ethanol, simmondsin has log ε 3.98 and simmondsin 2′-ferulate has log ε 4.31. There was some variation in retention time depending on the degree of deactivation of the column and the composition of the eluting solvent, acetonitrile–2-methoxyethanol. Typical LC conditions are summarized as follows: solvent, acetonitrile–2-methoxyethanol (97:3 to 94:6); flow rate, 1.0 mL/min, @ ~1000 psi; chart speed, 0.5 cm/min; temperature, ambient; detector, 220 nm, attenuation variable; retention times, simmondsin, 6.3 to 7.5 min, simmondsin 2′-ferulate, 3.7 to 4.7 min. A typical scan of seed meal extract is presented in Figure 1. Successful separations were also obtained on a 3.2 × 500 mm column packed with Porasil A using the 94:6 ratio solvent.

Thin-Layer Chromatography. The TLC plates were prepared using silica gel G (Merck) as the absorbant, drying in the oven at 100 °C for at least 20 min, and storing undesiccated. On development with ethyl acetate–ethanol (7:3), simmondsin has R_f ~0.35 and simmondsin 2′-ferulate has R_f ~0.68. Other developing solvents that work almost as well are chloroform–methanol (4:1), methylene chloride–methanol (6:1), and 100% isopropyl alcohol. Sim-

Table I. Composition of Jojoba Seeds and Hulls

compd	Apache 377 seeds, %	SCJP 977 seeds, %	seed hulls
oil[a]	50.2	53.8	0.7
meal, air dried[b,c]	49.0	44.0	
weight loss	0.8	2.2	
crude protein, N × 6.25	15.1	14.9	7.0
crude fiber	4.2	3.5	15.6
moisture	4.6	4.3	10.7
ash	1.6	1.4	4.4
carbohydrates			
reducing sugars	2.96	2.85	
nonreducing sugars	1.59	0.84	
total sugars, as invert	4.55	3.69	3.33
other, as polysaccharides	20.3	17.4	
simmondsin	2.34	2.27	0.19
simmondsin 2′-ferulate	0.75	0.36	

[a] Hexane extracted plus proximate analysis for crude fat. [b] Hexane extracted less proximate analysis for crude fat. [c] Amino acid composition of this meal is reported in Table II. Protein data reported in the literature was actually on a deoiled meal rather than a seed basis (NAS, 1975; Yermanos and Duncan, 1976).

mondsin 2′-ferulate fluoresces strongly under UV light (254 mm) and detects quickly and strongly in iodine vapor. Simmondsin detects slowly and weakly in iodine vapor. Both compounds char well with a 10% sulfuric acid spray followed by heat. High–low TLC (Grady et al., 1973) was used to monitor the meals and foliage for an estimate of their toxicant content, making visual comparisons with several known quantities. The minimum level of detection of simmondsin 2′-ferulate by fluorescence is about 0.5 μg. Simmondsin and simmondsin 2′-ferulate can be detected by sulfuric acid charring at a minimum level of about 1.5 μg. These limits apply to pure toxicants.

Infrared Spectra. The plant parts were deoiled with hexane in a Soxhlet extractor, and an infrared spectrum of the residual oily material was compared with that of pure jojoba oil, using a Perkin Elmer Model 421 infrared spectrophotometer.

RESULTS AND DISCUSSION

The composition of jojoba seeds from Arizona (Apache 377) and Southern California (SCJP 977) is reported in Table I and Table II. Oil content is about 50% and total crude protein content of the seeds is about 15%. The total reducing plus nonreducing sugars are about 4% of the seed, compared with 6.5% in an earlier analysis (Wells, 1955). Amino acid composition of the two seed samples were similar and comparable to prior reported data (Yermanos, 1974). Tests for glucose were positive and a test for starch in seeds was negative. The hulls, a component in commercially expressed jojoba seed meal, contained 7.0% crude protein and 3.3% total sugars.

Most notable here is the toxicant level in the seeds. The simmondsin levels in both samples are ~2.3% of the whole seed. Simmondsin 2′-ferulate content of the Apache 377 seeds at 0.75% was somewhat higher than that of the SCJP 977 seeds at 0.36%. This is a very high level of toxic material in an oil seed and is comparable to the high levels of glucosinolates found in Crambe seed (Van Etten and Wolff, 1973). Simmondsin added to the normal diet of rats at a 0.15% level caused the rats to reduce their feed intake (Booth et al. 1974).

Jojoba foliage contains significant amounts of simmondsin, ~0.2% in the leaves and hulls and ~0.7% in the twigs as summarized in Table III. This high toxicant level may also make these parts of the plant unsuitable for grazing animals in the food chain. Even woody tissue from a branch of an old bush at least 60 years old contained simmondsin. Simmondsin 2′-ferulate was not found in leaves, hulls, twigs, core wood, or male inflorescence. However, the hulls, leaves, twigs, and male inflorescence all contained a small amount of hexane-extractable oily material whose infrared spectra are similar to that of pure jojoba oil. Methods to detoxify the meal to make it suitable as an animal feed and nutritional studies on the processed meals are now underway. The isolation and purification of two of the minor toxicants (Elliger et al., 1974b) is also in progress.

Table II. Amino Acid Composition of Deoiled Jojoba Seed Meal[a]

amino acids[b]	Apache 377, %	SCJP 977, %
lysine	1.05	1.11
histidine	0.486	0.493
arginine	1.56	1.81
aspartic acid	2.18	3.11
threonine	1.14	1.22
serine	1.04	1.11
glutamic acid	2.40	2.79
proline	0.958	1.10
glycine	1.50	1.41
alanine	0.832	0.953
valine	1.10	1.19
methionine	0.186	0.210
isoleucine	0.777	0.866
leucine	1.46	1.57
tyrosine	1.04	1.05
phenylalanine	0.919	1.07
cystine and cysteine	0.791	0.519
tryptophan	0.492	0.559

[a] On exposure of this meal to the atmosphere, differences in humidity cause variations in water content as much at 5%. This may affect some of the analyses accordingly. [b] Actually the amino acid residue rather than the whole amino acid as percent of the meal.

Table III. Simmondsin and Oil in Plant Parts

plant part	oily material,[a] %	simmondsin, %
core wood[b]		0.45
leaves[c]	2.0	0.19
leaves[d]		0.23
twigs[c]	1.1	0.63
twigs[d]		0.71
influorescence[c]	1.9	0.22

[a] Hexane extracted, with infrared spectrum similar to jojoba oil. [b] Woody tissue from the inner part of a branch with an 18-cm circumference growing naturally near Aguanga in Southern California. [c] Male leaves, twigs, and influorescences (prior to anthesis) obtained from a plant North of Tucson, Arizona. [d] Unknown sex.

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A High-Performance Liquid Chromatographic Method for the Quantitation of Hesperidin in Orange Juice

Hesperidin was resolved from filtered orange juice by high-performance liquid chromatography (LC) using a micro C-18 column and eluting with a water–acetonitrile system. Detection was accomplished at 285 nm. This procedure improves on present hesperidin analysis by shortening analysis time and enhancing analytical confidence.

Hesperidin is the 7β-rutinoside of 2*S*-hesperetin (Horowitz, 1964). This compound is the predominant flavonoid glycoside in Florida orange juice. Hesperidin is tasteless and does not contribute to juice quality; however, its concentration in juice may be used as a measure of extraction pressure and related juice quality. Hesperidin has been reported to exert an apparent regulatory action on erythrocyte concentration and tissue perfusion in humans (Robbins, 1975).

Methods for the isolation and measurement of hesperidin have been reviewed by Kefford and Chandler (1970). The object of this work was to see if the technique of LC could improve on the present analyses for hesperidin. The following procedure was developed and used in this laboratory.

MATERIALS AND METHODS.

Apparatus. A Model ALC 202 high-performance liquid chromatograph with a Model 6000 A pump and U6K injector (Water Associates, Milford, Mass.) was used. The recorder was a Texas Instrument Servo/Riter II 2-pen. A Schoeffel UV-visible liquid chromatography analyzer Model SF 770 (Schoeffel Instrument Corp., Westwood, N.J.) was the detector. A Perkin-Elmer Model 457 infrared spectrophotometer equipped with a Wilks micro sampling system Model 45A and KRS–5, 2-mm crystal was used to help identify hesperidin. Peak areas were determined with a Spectra-Physics integrator (minigrator, Spectra-Physics, Santa Clara, Calif). A Waters Associates sample clarification kit with 1.2 or 0.45 Millipore aqueous filter system was used.

Column. A Waters Associates 30-cm × 4-mm i.d. reverse-phase μ Bondapak C-18 column (octadecyltrichlorosilane chemically bonded to <10 μ Porasil packing) was used.

Reagents. The eluting system was water–acetonitrile (80:20, v/v). The system was degassed with an ultrasonic bath. The crude hesperidin was obtained from Sigma (St. Louis, Mo.).

Sample Preparation. Fresh hand-squeezed, processed single-strength orange juice or reconstituted concentrate was filtered through glass wool. The resulting filtrate was refiltered through the Millipore clarification system.

High-Performance Liquid Chromatographic Resolution and Quantitation of Hesperidin. An aliquot (10–50 μL) of the above filtered juice was injected onto the column with a flow rate of 1.5 mL/min. Detection was accomplished at 285 nm with 0.1 absorbance unit full scale. Integration was conducted at an attenuation of 1.0, peak width setting of 35 and slope sensitivity of 150. The recorder chart speed was 12 in./h.

The quantity of hesperidin in unknown samples was determined from a linear regression equation. This equation was obtained from ten standard samples of hesperidin in dimethylformamide over the range of 1.0 to 10.0 μg. These samples were eluted isocratically and detected under the above conditions.

Percent Recovery and Precision. The reliability of the procedure was determined by fortifying five identical base samples of orange juice with sufficient hesperidin to provide concentrations of 100 to 600 ppm. The native hesperidin in the base sample was previously determined by this LC procedure.

The precision of the method was determined by analyzing five aliquots from an orange juice sample containing hesperidin.

Identification of Hesperidin. Recycle of the peak labeled hesperidin in Figure 1 showed this peak to be composed of only one constituent after seven passes through the column. The eluate corresponding to this peak area was collected and concentrated by freeze-drying. The identity of this fraction as hesperidin was based on the flavanone test (Horowitz, 1957), characteristic UV spectra (Jurd, 1962), and comparison of its infrared spectrum with

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