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Journal of Ethnopharmacology 96 (2005) 521–528

Journal of ETHNO-PHARMACOLOGY

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The value of plant collections in ethnopharmacology: a case study of an 85-year-old black cohosh (*Actaea racemosa* L.) sample

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Received 21 July 2004; received in revised form 27 September 2004; accepted 27 September 2004
Available online 23 November 2004

Abstract

Ethnopharmacological collections of plants used in traditional medical systems are a valuable but often underappreciated resource for scientific investigation. These collections contain many samples of plants currently employed in herbal and pharmaceutical medicine, and questions on stability and storage life can be examined using these historic collections as vouchers. A sample of black cohosh (*Actaea racemosa* L.), collected in 1919 by the physician and plant explorer Henry Hurd Rusby, was recently identified in the collections of The New York Botanical Garden and analyzed for its triterpene glycosidic and phenolic constituents qualitatively and quantitatively by high-performance liquid chromatography–photodiode array detector (HPLC–PDA) and liquid chromatography–mass spectrophotometry (LC–MS). A comparison of the triterpene glycosidic and phenolic constituents of the 85-year-old plant sample with those of a modern collection of *Actaea racemosa* showed the similarity of the two samples, confirming the stability of the older sample, despite its curation over the years under a variety of conditions. Quantitative analyses indicated that both plant samples have similar amounts of the four major triterpene glycosides, but the total amount of the six major phenolic constituents measured in the 85-year-old plant material is lower than the amount measured in the modern plant material. Methanol extracts of the two plant materials were tested for their antioxidant activity, and both extracts showed similar antioxidant activity.
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Keywords: Black cohosh; *Actaea racemosa*; *Cimicifuga racemosa*; Triterpene glycosides; Phenolics; Stability

1. Introduction

One resource not well investigated in the field of ethnopharmacology is the collections that were made during the late 1800s and early 1900s when explorers traversed the globe in search of novel and useful plants, particularly medicinal plants. These collections were used in pharmacology and pharmacognosy classes, to teach students about the pharmacopoeia of the time. Many collections ended up as displays at museums, botanical gardens, and universities, educating and fascinating the public about the importance of plants to their lives. Currently, the deterioration, deaccessoning, and "orphaning" of many underfunded collections, as is the case with many biodiversity voucher collections, is a serious threat to these collections (Miller et al., 2004).

We suggest that ethnopharmacological collections from past centuries can serve contemporary research as voucher collections to answer a variety of questions. These include issues in compound stability, diversity, distribution, and change. They represent a snapshot in time of the chemistry and utilization by past cultures that can never be recreated, and thus should be recognized and supported for their value to science.

Henry Hurd Rusby (1855–1940) was a physician and botanist who explored and collected plants in North and South

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doi:10.1016/j.jep.2004.09.049

America throughout his productive career. His Latin American fieldwork took him to Bolivia, Brazil, Chile, Columbia, Ecuador, Mexico, Peru, and Venezuela (Williams and Fraser, 1992). With 3 days' notice, shortly after receiving his medical degree, he led a 2-year expedition (1885–1886) to South America sponsored by Parke, Davis, and Co. in search of bioactive plants.

A black cohosh sample, collected by Rusby in 1919, was identified recently in the collections of The New York Botanical Garden (NYBG), where it had been exhibited and/or stored for 85 years under a variety of environmental conditions, including those not normally considered favorable to the preservation of chemical compounds. Rusby, known as the father of economic botany at The NYBG, made extensive collections of the medicinal and other useful plants of his time, including samples of medicinal plants available on the commercial market (Williams and Fraser, 1992). The black cohosh sample analyzed in this paper was typical of the latter, a commercially available dried root and rhizome powder, purchased on the open market and stored in a glass bottle. As we are currently working on aspects of the phytochemistry, ethnobotany and clinical application of black cohosh (Kennelly et al., 2002; Lupu et al., 2003; Einbond et al., 2004), we were curious to see how this 85-year-old sample differed from contemporary material of the same species, particularly with regard to stability.

The roots and rhizomes of *Actaea racemosa* L. (black cohosh) (syn. *Cimicifuga racemosa*) have a long and diverse history of medicinal use in North America. Traditionally, Native Americans and early colonists used its root to treat a variety of conditions including general malaise, malaria, rheumatism, abnormalities in kidney function, sore throat, menstrual irregularities, and assist with childbirth (Barton, 1798; Rafinesque, 1828; Low Dog et al., 2003). In the past 40 years, the plant has been used as an herbal medicine in North America and Europe primarily for the treatment of symptoms related to menopause (Borrelli et al., 2003). There are a variety of black cohosh products currently available to consumers, and considerable research on this plant has been reported, including at least four placebo and/or treatment controlled clinical trials (Warnecke, 1985; Stoll, 1987; Lehmann-Willenbrock and Riedel, 1988; Jacobson et al., 2001), but none of these trials has been conducted for more than 6 months (Kronenberg and Fugh-Berman, 2002). Retail sales of black cohosh herbal products in 2002 ranked ninth in terms of the U.S. botanical products' market (Blumenthal, 2003).

The chemical constituents of black cohosh include two classes of secondary products, triterpene glycosides and phenolics. Previous research on the chemical constituents of black cohosh has resulted in the isolation and identification of more than 40 triterpene glycosides (Panizzi and Corsano, 1962; Bedir and Khan, 2000, 2001; Shao et al., 2000; Hamburger et al., 2001; Wende et al., 2001; Chen et al., 2002a,c; Watanabe et al., 2002) and 14 polyphenolics (Kruse et al., 1999; Burdette et al., 2002; Chen et al., 2002b). Other constituents of black cohosh have been identified including a tannin with an unreported structure (Finnemore, 1909), and an oleanol triterpene glycoside (Suntry Ltd., 1984). Fig. 1 shows

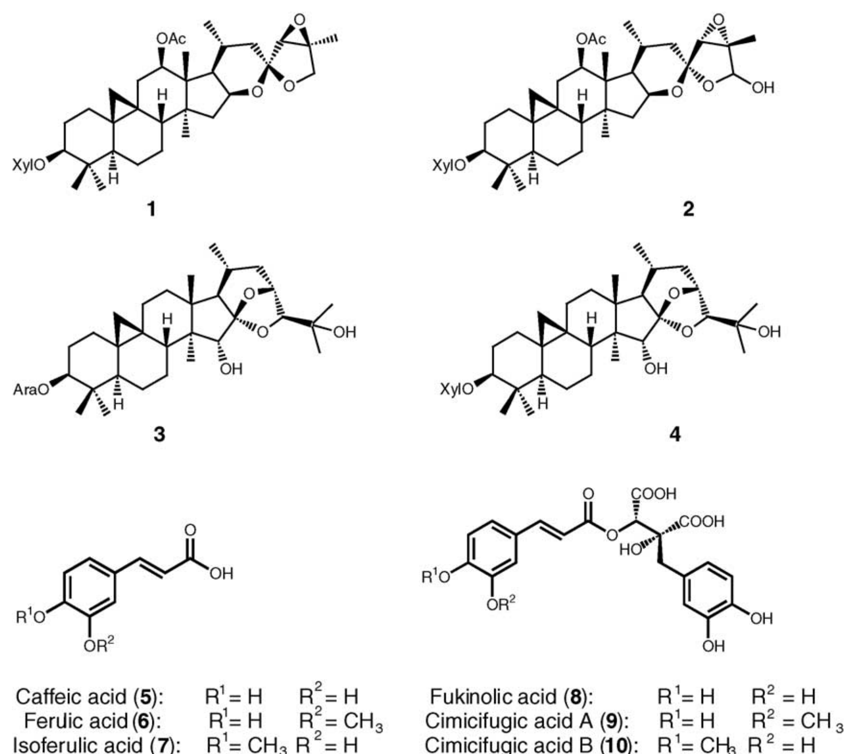

Fig. 1. Structures of selected triterpene glycosides and phenolics identified from black cohosh.

four triterpene glycosides (**1–4**) and six phenolics (**5–10**) that have been reported in this plant, and these 10 compounds have been analyzed in this work. It is not currently known if these or other black cohosh constituents are most relevant to the present clinical use of the plant for menopausal symptoms.

This 85-year-old black cohosh material was compared with a contemporary sample for similarities and differences in their phytochemical profiles, and to determine whether constituents are stable when the plant is stored for an extended time. The samples were compared using high-performance liquid chromatography–photodiode array detector (HPLC–PDA) and liquid chromatography–mass spectrophotometry (LC–MS) methods to analyze the triterpene glycosides and phenolic compounds qualitatively and quantitatively.

2. Material and methods

2.1. General experimental procedures

The samples and standards were analyzed using HPLC on a Waters 2695 separations module (Milford, USA) equipped with a 996 photodiode array detector (PDA), and operated with Empower software. Separations were carried out on a Waters C_{18} column (3.9 mm × 150 mm, 5 μm) for the triterpene glycosides, and a Phenomenex Aqua C_{18} column (4.6 mm × 250 mm, 5 μm) for the phenolics at ambient temperature with a flow rate of 1.0 ml/min. The sample volume injected was 10 μl and data were analyzed at 203 nm for triterpene glycosides, and 320 nm for phenolic compounds. According a published method (He et al., 2000), the mobile phase for the analysis of triterpene glycosides consisted of a step gradient starting with 5% (v/v) acetonitrile (solvent A) in water (B) and increasing to 75% acetonitrile over 55 min. The gradient profile was: 0–18 min: 5–28% A; 18–36 min: 28–35% A; 36–45 min: 35–55% A; 45–55 min: 55–75% A. We developed a solvent system for the analysis of the phenolic constituents composed of acetonitrile (A) and 10% aqueous formic acid (B) using a step gradient elution of 5–15% A at 0–15 min, 15% A at 15–20 min, 15–50% A at 20–50 min, and 50–100% A at 50–55 min. The UV–vis spectra were recorded from 200 to 500 nm. A similar method has recently been published by others (Li et al., 2003). Preparative HPLC was carried out using a Waters 600 controller with a Waters 486 tunable absorbance detector and Waters Empower software with a Phenomenex Nucleosil C_{18} column (21.1 mm × 250 mm, 10 μm) and an isocratic solvent system of 0.1% acetic acid/MeCN (80:20, v/v), a flow rate of 5 ml/min, column at room temperature and 60 min run time.

Mass spectra were recorded on a LCQ Mass Spectrometer (ThermoFinnigan, San Jose, USA) equipped with an atmospheric pressure chemical ionization (APCI) source. APCI was performed with the discharge current at 5 μA. The vaporizer and capillary temperatures were set to 450.0 and 150.0 °C, respectively. The sheath gas and auxiliary gas, both nitrogen, had flow rates of 80 and 10 units, respectively. A mass range of 50–1000 amu was scanned.

2.2. Solvents and chemicals

HPLC grade acetonitrile (J.T. Baker, Phillipsburg, USA), chloroform (Aldrich, Milwaukee, USA), and dimethyl sulfoxide (Aldrich, Milwaukee, USA) were used for sample preparation and HPLC analysis. Reagent grade methanol (E. Merck, Darmstadt, Germany), formic acid (E. Merck, Darmstadt, Germany), and sodium hydroxide (Fisher Scientific, Fair Lawn, USA) were used for the extraction and separation of compounds **7–10**, which were utilized as the authentic references. The absorbents for open column chromatography were octadecyl (C_{18} 40 μm) (J.T. Baker, Phillipsburg, USA) and Diaion HP-20 (Supelco, Bellefonte, USA).

2.3. Plant materials and sample preparation

The 85-year-old black cohosh material was a commercial sample presented to Rusby by Parke, Davis, and Company, on July 3, 1919. It is of unknown provenance but there is a published note that the material was from Montclair Heights, New Jersey (Rusby, 1921). The material was stored in the collections of The NYBG with an identification number of H.H. Rusby #4922. For many years it was exhibited in The NYBG Economic Museum, an exhibit that attracted much public interest and contained thousands of other collections made by Rusby. The Economic Museum opened in 1899 and closed in the 1930s, when the exhibit was dismantled. The bottle containing powdered black cohosh roots and rhizomes was wrapped in newspaper, placed inside a cardboard box and stored for many decades in a moist basement environment.

A portion of the powdered black cohosh plant material (1.0208 g) was extracted with 30 ml of 80% $MeOH/H_2O$ at ambient temperature for 12 h and then filtered. The resulting residue was extracted with 80% $MeOH/H_2O$ three more times. The filtrates were combined and evaporated in vacu to give a brown extract (184.4 mg).

A portion of the 80% MeOH extract (101.0 mg) was dissolved in 15 ml 0.5% NaOH water solution. The solution was sonicated for 10 min, and then transferred into a 120 ml separatory funnel, and partitioned with chloroform (40 ml) by shaking vigorously for 2 min. The NaOH aqueous solution was partitioned three more times using 40 ml of chloroform each time. All chloroform fractions were combined and evaporated under reduced pressure. The resulting residue (25.6 mg) was dissolved in 2 ml of DMSO, and filtered through a 0.45 μm nylon membrane. The filtered sample was then analyzed for triterpene glycosides by HPLC.

A portion of the 80% MeOH extract (7.98 mg) was dissolved in 2.00 ml of 70% $MeOH/H_2O$ solution. The solution was sonicated for 1 min, and then passed through a 0.45 μm nylon membrane filter. This solution was analyzed for phenolic compounds by HPLC.

The modern black cohosh roots and rhizomes were collected in the northeast part of the United States and supplied by PureWorld Botanicals Inc. (South Hackensack, USA) with lot #9-2677 and item #02580-001.

Using the same method described above, 10.06 g modern black cohosh roots and rhizomes were extracted to yield a brown extract (1.50 g). A portion of the extract (400.1 mg) was used to produce a sample for the analysis of triterpene glycosides, and 132.8 mg residue was obtained and dissolved in 10 ml of DMSO. Also 8.57 mg of extract was dissolved in 2.00 ml 70% MeOH/H_2O solution to prepare a sample for the analysis of phenolic constituents.

2.4. Reference compounds

23-Epi-26-deoxyactein (**1**) (Chen et al., 2002c), formerly known as 27-deoxyactein, was purchased from ChromaDex (Santa Ana, USA) (lot #02-04130-103). Caffeic acid (**5**) and ferulic acid (**6**) were obtained from Sigma Chemical Co. (St. Louis, USA) (lot #22H3650 and 43H0100, respectively). Isoferulic acid (**7**, 97.37% purity), fukinolic acid (**8**, 95.98% purity), cimicifugic acid A (**9**, 90.07% purity), and cimicifugic acid B (**10**, 79.53% purity) were isolated from black cohosh extract by the following procedures. A dried ethanolic extract of black cohosh (PureWorld Botanicals Inc., USA) was redissolved in water, and then partitioned with hexane and *n*-butanol successively. The resulting *n*-butanol extract was further separated by Diaion HP-20 eluting with MeOH/H_2O (1:1, v/v). From fraction BHP-7 (1.03 g), isoferulic acid (**7**, 284.2 mg) was obtained as a crystal. The water solution was separated using Diaion HP-20 eluting with MeOH/H_2O (1:1, v/v) to give 5 fractions I–V. Fraction II (300.0 mg) was further separated by C_{18} column chromatography eluting with 0.1% acetic acid/MeCN gradient solvent system (95:5–80:20, v/v; 5% steps) to yield a total of 16 subfractions. Fukinolic acid (**8**, 42.6 mg) was obtained from subfraction 9 while cimicifugic acid A (**9**, 8.1 mg) was obtained from subfraction 13. Subfraction 16 was further separated by preparative C_{18} HPLC eluting with an isocratic solvent system 0.1% acetic acid/MeCN (80:20, v/v), and cimicifugic acid B (**10**, 2.8 mg) was obtained. The purities of compounds **7**–**10** were determined by HPLC–PDA at 320 nm.

2.5. DPPH assay

Reaction mixtures containing test samples (50 μl dissolved in DMSO) and 150 μl of a 400 μM DPPH ethanolic solution were incubated at 37 °C for 30 min in 96-well microtiter plates. Absorbance of the free radical abstracted DPPH was measured at 515 nm with a $VERSA_{max}$ tunable microplate reader (Molecular Devices, Sunnyvale, USA), and the percent inhibition was determined by comparison with DMSO control groups. A plot of the percent inhibition versus the concentration of sample at 515 nm was constructed, and IC_{50} values were determined to be the concentration of the sample required to scavenge 50% of DPPH free radicals. All experiments were performed in duplicate.

3. Results

The 85-year-old and modern black cohosh samples were analyzed and evaluated for both their triterpene glycosidic and phenolic constituents. The triterpene glycosides in HPLC–PDA spectrum appeared as small peaks and often were overlapped by other types of compounds. In order to partially purify the samples before HPLC–PDA analysis, a previously published solvent-solvent portioning scheme was employed for each black cohosh sample to obtain fractions enriched in triterpene glycosides (He et al., 2000). According to the HPLC analysis, the 85-year-old plant material possesses similar triterpene glycosides to those of the modern plant (Fig. 2A). The LC–MS spectra demonstrated a similarity of the triterpene glycosides in the two samples; no difference in peaks was found in the LC–MS spectra of the two samples (Fig. 2B). However, the relative content of each triterpene glycoside differed between the 85-year-old black cohosh sample and the modern sample.

Four of the major black cohosh constituents, 23-epi-26-deoxyactein (**1**), actein (**2**), cimiracemoside C (**3**), and cimigenoside (**4**), were analyzed quantitatively in this study. The triterpene glycosides were identified by comparing their retention time (t_R) and mass spectra with authentic standards via LC–MS, and these data agreed with those reported previously (He et al., 2000). Compounds **1** and **2** were found to overlap at t_R 33–34 min in HPLC–PDA and LC–MS spectra in our experiments, but the two compounds could be identified by two different fragment patterns in the mass spectra (He et al., 2000). Compounds **1** and **2** were further analyzed by LC–MS individually, and these compounds displayed peaks with indistinguishable t_R. Quantitative analyses of the triterpene glycosides were carried out by both HPLC–PDA and LC–MS methods. The amounts of these four triterpene glycosides were calculated by comparison of each peak area with that of standard, 23-epi-26-deoxyactein (**1**). The total amount of the four triterpene glycosides of the 85-year-old sample was quantified by HPLC–PDA and LC–MS to be 4.43–4.73% while the amount for the modern sample was 4.06–5.18% (Table 1).

For the analysis of phenolic constituents of black cohosh, a new HPLC–PDA method was developed. Both the 85-year-old and modern black cohosh samples exhibited similar HPLC–PDA chromatograms at 320 nm for the phenolic constituents (Fig. 3A and B). In the HPLC–PDA chromatograms, each sample clearly displayed six strong peaks which were determined to be caffeic acid (**5**), ferulic acid (**6**), isoferulic acid (**7**), fukinolic acid (**8**), cimicifugic acid A (**9**), and cimicifugic acid B (**10**) by comparing the t_R, UV absorption, and LC–MS data with those of six reference phenolic standards (**5**–**10**). The HPLC–PDA chromatogram for the mixture of the six reference phenolic compounds is shown in Fig. 3C.

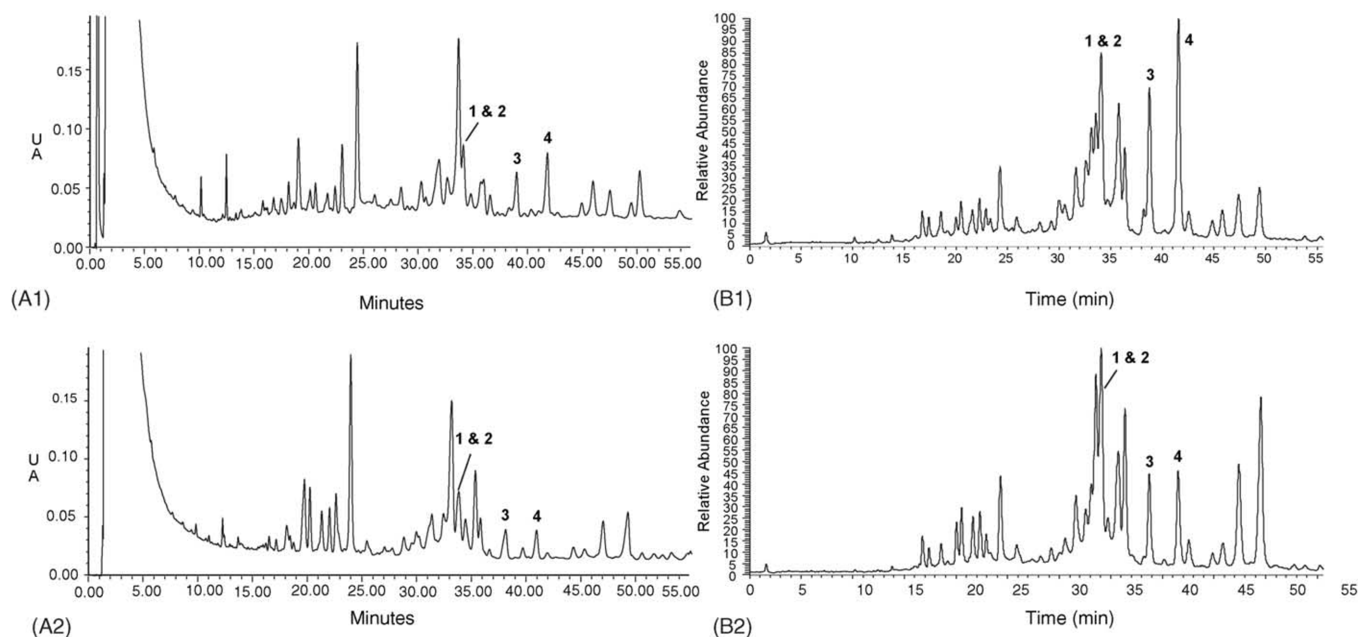

Fig. 2. HPLC–PDA and LC–MS spectra for the triterpene glycosides of two black cohosh samples. Spectra A: HPLC–PDA at 203 nm; spectra B: LC–MS TIC. Spectra 1 for the 85-year-old black cohosh plant material; spectra 2 for the modern plant material.

Table 1
Amounts of four triterpene glycosides in the two black cohosh extracts

Samples	23-Epi-26-deoxyactein and actein[a] (**1** and **2**) (mg/ml)	Cimiracemoside C (**3**) (mg/ml)	Cimigenoside (**4**) (mg/ml)	Total amount (%)
HPLC–PDA analysis				
85-Year-old plant	0.9145 ± 0.035[b]	0.6228 ± 0.030	0.8524 ± 0.014	4.732
Modern plant	1.2073 ± 0.13	0.4525 ± 0.12	0.4147 ± 0.0079	5.185
LC–MS analysis				
85-Year-old plant	0.7877 ± 0.096	0.5903 ± 0.084	0.8595 ± 0.040	4.431
Modern plant	0.8911 ± 0.050	0.3572 ± 0.033	0.3751 ± 0.0083	4.058

[a] Actein and 23-epi-26-deoxyactein overlapped.
[b] Mean ± S.D. ($n = 4$ for the 85-year-old plant; $n = 3$ for the modern plant).

Using the HPLC–PDA method, the contents of the six phenolic compounds were quantified. The total amount of these phenolics of the 85-year-old sample was determined to be 4.25%, and the total amount of the phenolics for the modern sample 5.68%. Compounds **7–10** comprise the major phenolic constituents of the two black cohosh plant materials tested (Table 2).

The two black cohosh plant samples were also assayed for their antioxidant property. Both methanol extracts from the two samples showed a moderate activity in the DPPH

Table 2
Amounts of six phenolic compounds in the two black cohosh extracts determined by HPLC–PDA

Standards	85-Year-old plant		Modern plant	
	(mg/ml)	w/ext. (%)	(mg/ml)	w/ext. (%)
Caffeic acid (**5**)	$0.002564 \pm 8.49 \times 10^{-5}$[a]	0.06426	$0.007484 \pm 1.05 \times 10^{-4}$	0.1747
Ferulic acid (**6**)	$0.003572 \pm 4.08 \times 10^{-5}$	0.08952	$0.007348 \pm 1.09 \times 10^{-4}$	0.1715
Isoferulic acid (**7**)	$0.02001 \pm 2.75 \times 10^{-4}$	0.5015	$0.02740 \pm 2.19 \times 10^{-4}$	0.6394
Fukinolic acid (**8**)	$0.05348 \pm 3.64 \times 10^{-4}$	1.3404	$0.08345 \pm 2.25 \times 10^{-4}$	1.9475
Cimicifugic acid A (**9**)	$0.03635 \pm 3.62 \times 10^{-4}$	0.9110	$0.05262 \pm 1.93 \times 10^{-4}$	1.2280
Cimicifugic acid B (**10**)	$0.05343 \pm 5.11 \times 10^{-4}$	1.3391	$0.06513 \pm 1.62 \times 10^{-3}$	1.5200
Total amount (%)		4.2458		5.6811

[a] Mean ± S.D. ($n = 5$).

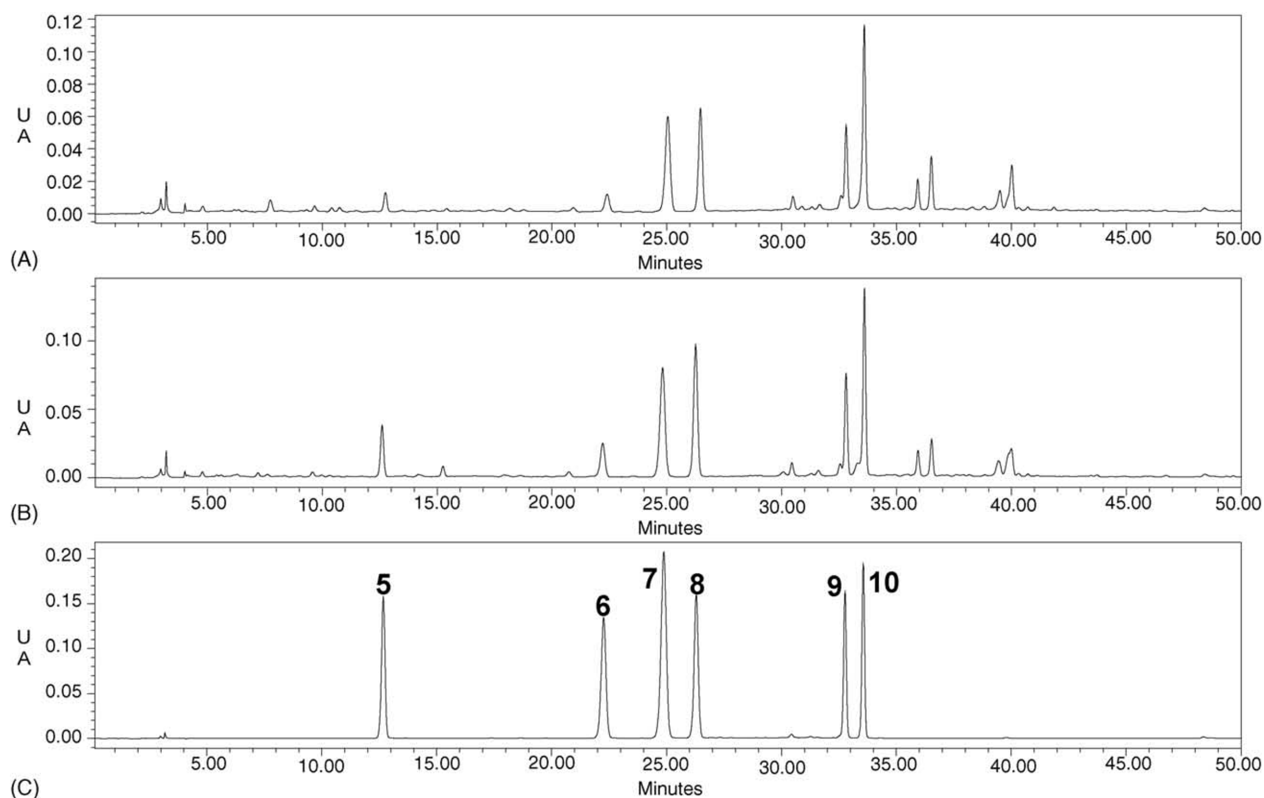

Fig. 3. HPLC–PDA (320 nm) spectra for the phenolic constituents. A: the 85-year-old black cohosh plant material; B: the modern plant material; C: the mixture of six phenolic reference compounds, caffeic acid (**5**), ferulic acid (**6**), isoferulic acid (**7**), fukinolic acid (**8**), cimicifugic acid A (**9**), and cimicifugic acid B (**10**).

assay. The IC_{50} values for the 85-year-old and modern plant materials were 125.47 and 137.99 μg/ml, respectively, which agreed with the DPPH assay published results for black cohosh (Burdette et al., 2002).

4. Discussion

Based on the HPLC–PDA and LC–MS spectra, the 85-year-old and modern samples were very similar qualitatively with respect to triterpene glycosidic and phenolic constituents. For example, in the LC–MS spectra of the triterpene glycosides (Fig. 2B), the number of peaks observed did not vary between the 85-year-old sample and the modern sample. This indicates the stability of these constituents in the 85-year-old black cohosh sample. Triterpene glycosides are generally regarded as stable compounds, except under extreme pH or those compounds with a free carboxylic group (Miyamoto et al., 1984; Tava et al., 2003). Triterpene glycosides of black cohosh are monodesmosides and it is not possible to interconvert glycosides by hydrolysis of the sugar portion of the glycoside. Therefore, the differences of the relative proportions of some triterpene glycosides between the 85-year-old and modern black cohosh samples may be caused by harvesting black cohosh in different seasons or different locales, developmental stages, or selection pressures. Phytochemical studies of several plant species have found different relative proportions of the same natural constituents across seasonal or geographical location or plant growth stage (Lobstein et al., 1991; Wang and Chen, 1999). Moreover, depending upon what extraction solvent is used, we have found (unpublished) that if the modern plant material is extracted using 80% methanol (as used in our studies) or 70% ethanol or 40% isopropanol (as used in commercial preparations of black cohosh), the values for triterpene glycosides are from 3.1 to 5.2%, and for the phenolic compounds from 5.2 to 5.8%. The 85-year-old black cohosh plant methanol extracted material had a proportion of the major triterpene glycosides (4.4–4.7%) similar to contemporary samples.

We found that when black cohosh is extracted using 80% methanol, 70% ethanol, and 40% isopropanol, the ratios between some major phenolics are consistent. For example the ratio of compounds **6** to **9** is 1:7–10, and the ratio of compounds **7** to **10** is 1:2–4. According to our research, the ratio of **6** to **9** in the 85-year-old sample is 1:10.2 and in the modern sample 1:7.2. Likewise, the ratio of **7** to **10** in the 85-year-old sample is 1:2.7, and in the modern sample 1:2.4. Both ratios of the major phenolics in the 85-year-old sample are similar to those in the modern sample, suggesting that the different levels of the six major phenolics between the 85-year-old and the modern samples were not caused by the decomposition of some major phenolic constituents. Compounds **6** and **7** possess chemical structures significantly different from those of compounds **9** and **10** in both molecular weight and spatial

configuration, and a published report also indicated that the antioxidant activities of compounds **6** and **7** were significantly different from those of compounds **9** and **10** (Burdette et al., 2002). Therefore, it is unlikely that compounds **6** and **7** would decompose or convert into other constituents at the same rate as compounds **9** and **10** did. Moreover, comparing the HPLC–PDA chromatograms (extracted from 260 to 380 nm) of the two samples, no difference in the major peaks was detected, thereby suggesting the stabilities of the major phenolic constituents of the 85-year-old black cohosh sample. Thus the differences in the amount of the six major phenolics between the 85-year-old and the modern samples may be caused by a variety of other factors, such as the genetic or metabolic properties of the plant (Taiz and Zeiger, 1991), the season or time of day that the plants are harvested, the plant growing conditions such as type of soil and climatic conditions (Yang et al., 1989; Lobstein et al., 1991; Wang and Chen, 1999), the age of the plant (Yang et al., 1989; Wang and Chen, 1999), and the method for processing the plant material (Huang et al., 1999; Wang, 1999).

The 85-year-old black cohosh material has an overall phytochemical profile remarkably similar to the modern sample, indicating that black cohosh can be stored in the form of dry plant material for an extended time. This result may be helpful for the study of ancient medical practices, as well as to current manufacture of black cohosh products.

It has been reported that some commercial black cohosh products contain *Cimicifuga foetida* L. instead of *Actaea racemosa*. A major phytochemical difference between the two extracts is that cimiracemoside C (**3**) can only be detected in the product manufactured from *Actaea racemosa* (He et al., 2000). From the HPLC–PDA and LC–MS spectra, the presence of cimiracemoside C (**3**) in the 85-year-old black cohosh sample was observed in the present study, which suggests that the 85-year-old black cohosh plant material is not *Cimicifuga foetida*.

With regard to phytochemical stability, a similar study was conducted on a 106-year-old sample of *Banisteriopsis caapi* (Spruce ex Griseb.) C.V. Morton originally collected by British botanist Richard Spruce. It was found that the Spruce material consisted only of the β-carboline alkaloid, harmine, while the modern material contained harmine, along with harmaline and tetrahydroharmine (Schultes et al., 1969). The authors of this study concluded that, "It is open to question whether the stems sent home by Spruce in 1853 from the beginning contained only harmine or perhaps more likely that harmaline and tetrahydroharmine have with time been transformed into the chemically more stable aromatic β-carboline, harmine" (Schultes et al., 1969).

Though the result of this work on the historic Spruce material differs from our study of historic Rusby material, it represents an example of how the examination an old plant sample can be used to understand the stability of medicinal plant products. These studies of historic plant material suggest that stability of phytochemical constituents over time may vary greatly. Given that many commercial botanical products currently sold have expiration dates of 2–3 years following manufacturing, are stored in light-reducing bottles, and often come with silica gel inserts, our work suggests that black cohosh plant material has the potential to be stored for longer periods of time without major changes in its triterpenoid and phenolic profiles.

Studying other such historic materials could also provide information as to the stability of compounds and on evolutionary and genetic changes. The NYBG is currently re-curating the Rusby Collection in order that it can be preserved according to contemporary museum collection standards, and once again be available as a scientific resource.

Acknowledgements

We are grateful to the following persons at The NYBG: Dr. Richard Rauh for his tireless efforts in curating the Rusby collections; Ms. Daphne Christopher for locating the sample and creating the exhibit that brought this particular accession to the attention of the authors; and Ms. Jan W. Stevenson for coordinating the events and exhibits involving the Rusby materials. Dr. Michael J. Balick's activity on this project was supported by a MetLife Fellowship. The authors are thankful to PureWorld Botanicals Inc. for providing the modern black cohosh plant material. We thank Dr. Kan He, PureWorld Botanicals Inc., for checking the purity of a standard. We dedicate this work to the memory of our friend and colleague, late Ms. Natalie Koether, former CEO of PureWorld Botanicals Inc. We are also grateful to the Edith C. Blum Foundation for its contribution. Part of this work was supported by NIH-SCORE award S06GM08225. This project was made possible by grant #P50-AT00090 from the National Institute of Health–National Center for Complementary and Alternative Medicine (NIH–NCCAM). Its contents are solely our responsibility and do not necessarily reflect the official views of NIH–NCCAM.

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