gold concentrations in the ultrafiltrates were not statistically different ($p > 0.05$) from the corresponding concentrations prior to filtration. Hence, no corrections for nonspecific binding were warranted. Gold was almost 100% bound to bovine serum albumin (Table I). These results indicate that gold may be bound exclusively to the albumin fraction of plasma, as was reported earlier (2, 3, 9). However, more definite conclusions can be made only after studies in the presence of other plasma proteins. The overall values for the percent of gold bound to 2 ($n = 35$) and 4% ($n = 15$) bovine serum albumin were 98 ± 1.6 and 99 ± 1.0% (mean ± *SD*), respectively.

Two factors that often determine the extent of binding are the concentrations of the drug and of the protein. The gold concentrations studied were selected to include the plasma concentrations of the drug encountered in chrysotherapy (2, 6, 16, 24, 25). While there is a paucity of data regarding the extent of hypoalbuminemia in arthritis patients, a recent study observed serum albumin levels as low as 2.47 g/100 ml (11). The results of the present work show that, in the clinically important range, the extent of gold binding was independent of gold and bovine serum albumin concentrations. The validity of these results in the *in vivo* situation in humans remains to be examined.

Interaction with Salicylic Acid—Salicylic acid did not compete with gold for binding sites on bovine serum albumin (Table II). Since the sequence of addition of salicylic acid had no significant effect on the affinity of gold for bovine serum albumin, inhibition of a noncompetitive nature also can be ruled out. Similarly, gold did not interfere with salicylate binding; salicylate was 62–70% bound to bovine serum albumin in the absence and presence of gold (2–10-μg/ml range). During aspirin therapy, plasma concentrations of salicylate range from 50 to 200 μg/ml (26). The *in vitro* results suggest that mutual displacement of gold and salicylic acid from plasma proteins may not be a serious drug interaction in arthritis management.

The results of this study show that clinically encountered plasma concentrations of gold, salicylate, and albumin are unlikely to cause statistically significant alterations in the extent of protein binding of these two drugs. However, for drugs that are bound extensively to plasma protein as is gold, minor binding alterations may be clinically significant due to the resulting dramatic changes in the free concentration. For instance, a 1% decrease in the binding of gold (assumed to be 99% bound) results in a twofold (1–2%) increase in the free concentration of gold. Such changes, although extremely difficult to document experimentally, could cause gold toxicity in arthritic patients.

REFERENCES

(1) "The Pharmacological Basis of Therapeutics," 5th ed., L. S. Goodman and A. Gilman, Eds., Macmillan, New York, N.Y., 1975, p. 931.

(2) B. R. Mascarenhas, J. L. Granda, and R. H. Freyberg, *Arthritis Rheum.*, **15**, 391 (1972).

(3) E. G. McQueen and P. W. Dykes, *Ann. Rheum. Dis.*, **28**, 437 (1969).

(4) M. Harth, D. S. M. Haines, and D. C. Bondy, *Am. J. Clin. Pathol.*, **59**, 423 (1973).

(5) J. S. Lawrence, *Ann. Rheum. Dis.*, **20**, 341 (1961).

(6) A. Lorber, R. A. Bovy, and C. C. Chang, *Nature (New Biol.)*, **236**, 250 (1972).

(7) P. M. Smith, E. M. Smith, and N. L. Gottilieb, *J. Lab. Clin. Med.*, **82**, 930 (1973).

(8) N. Simon, *Science*, **119**, 95 (1954).

(9) D. S. Campion, R. Olsen, A. Bohan, and R. Bluestone, *J. Rheumatol. (Suppl. 1)*, **1**, 112 (1974).

(10) R. W. Mason, *Pharmacology*, **15**, 536 (1974).

(11) H. Kamel, D. H. Brown, J. M. Ottaway, and W. E. Smith, *Analyst*, **101**, 790 (1976).

(12) J. D. Davis, R. A. Turner, R. L. Collins, I. R. Ruchte, and J. S. Kaufmann, *Clin. Pharm. Ther.*, **21**, 52 (1977).

(13) J. F. Goodwin and A. J. Bollet, *J. Lab. Clin. Med.*, **55**, 965 (1960).

(14) A. A. Dietz and H. M. Rubinstein, *Ann. Rheum. Dis.*, **32**, 124 (1973).

(15) G. D. Christian, *Clin. Chem.*, **11**, 459 (1965).

(16) A. Lorber, R. L. Cohen, C. C. Chang, and H. E. Anderson, *Arthritis Rheum.*, **11**, 170 (1968).

(17) J. V. Dunckley, *Clin. Chem.*, **17**, 992 (1971).

(18) F. J. M. J. Maessen, F. D. Posma, and J. Balke, *Anal. Chem.*, **46**, 1445 (1974).

(19) J. Agget and T. S. West, *Anal. Chim. Acta*, **55**, 349 (1971).

(20) J. Agget, *ibid.*, **63**, 473 (1973).

(21) J. P. Matousek and B. J. Stevens, *Clin. Chem.*, **17**, 636 (1971).

(22) S. M. Singhvi, A. F. Heald, H. H. Gadenbusch, M. E. Resnick, L. T. Difazio, and M. A. Leitz, *J. Lab. Clin. Med.*, **89**, 414 (1977).

(23) D. T. Plummer, "An Introduction to Practical Biochemistry," McGraw-Hill, Berkshire, England, 1971, p. 156.

(24) A. Lorber, *Clin. Pharmacokinet.*, **2**, 127 (1977).

(25) J. T. Sharp, M. D. Lidsky, J. Duffy, H. K. Thompson, Jr., B. D. Person, A. F. Masri, and A. A. Andrianakos, *Arthritis Rheum.*, **20**, 1179 (1977).

(26) M. Mayersohn, *J. Am. Pharm. Assoc.*, **NS 17**, 107 (1977).

ACKNOWLEDGMENTS

Presented at the Basic Pharmaceutics Section, APhA Academy of Pharmaceutical Sciences, Phoenix meeting, November 1977.

Supported in part by Program 821, Veterans Administration Center, Fargo, ND 58102.

GLC Analysis of Poison Ivy and Poison Oak Urushiol Components in Vegetable Oil Preparations

MAHMOUD A. ELSOHLY ˣ and CARLTON E. TURNER

Received August 6, 1979, from the *Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, University, MS 38677.* Accepted for publication November 26, 1979.

Abstract □ A procedure is described for the analysis of urushiol content of pharmaceutical preparations containing extracts of poison ivy (*Toxicodendron radicans*) and poison oak (*T. diversilobum*) in vegetable oils. The procedure involves extraction of the urushiols from the oily solutions using 90% methanol in water followed by GLC analysis of the extracts. Recoveries of both poison ivy and poison oak urushiols from solutions in corn oil, olive oil, sesame seed oil, and cottonseed oil were calculated. Correlation coefficients (r^2) ranged from 0.97 to 1.00, and the coefficients of variations ranged from 3.08 to 7.90%.

Keyphrases □ Urushiols—GLC analysis, pharmaceutical preparations containing poison ivy and poison oak extracts in vegetable oils □ GLC—analysis, urushiols in pharmaceutical preparations containing poison ivy and poison oak extracts in vegetable oils □ Poison ivy—urushiol components in vegetable oil preparations, GLC analysis

Contact dermatitis due to poison ivy (*Toxicodendron radicans*) and other related *Anacardiaceae* species [*e.g.*, poison oak (*T. diversilobum*) and poison sumac (*T. vernix*)] is a major problem among outdoor workers in the

0022-3549/80/0500-0587$01.00/0

© 1980, American Pharmaceutical Association

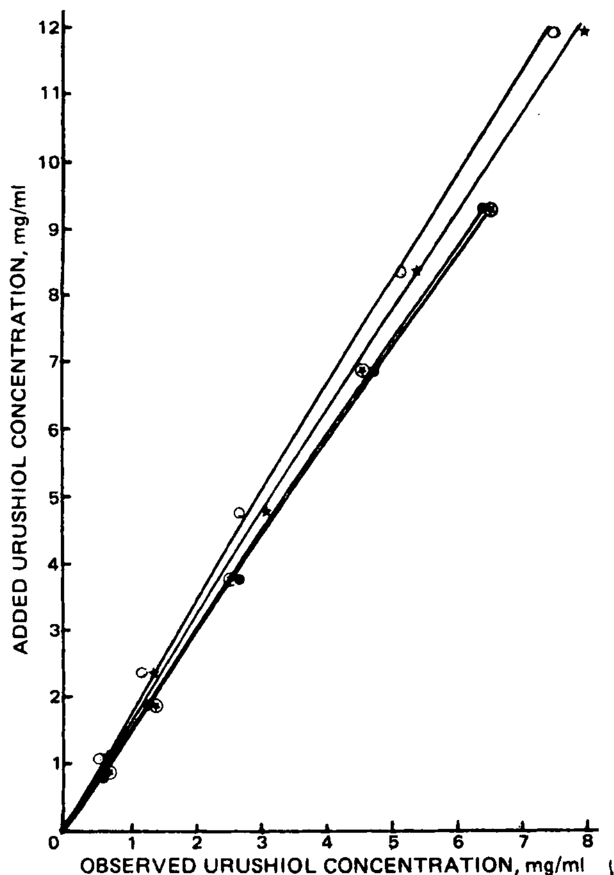

Figure 1—*Recovery curves for the extraction of urushiol from solutions of poison ivy extract in corn oil (⊛), olive oil (●), sesame seed oil (★), and cottonseed oil (○) using a methanol–water (9:1) mixture.*

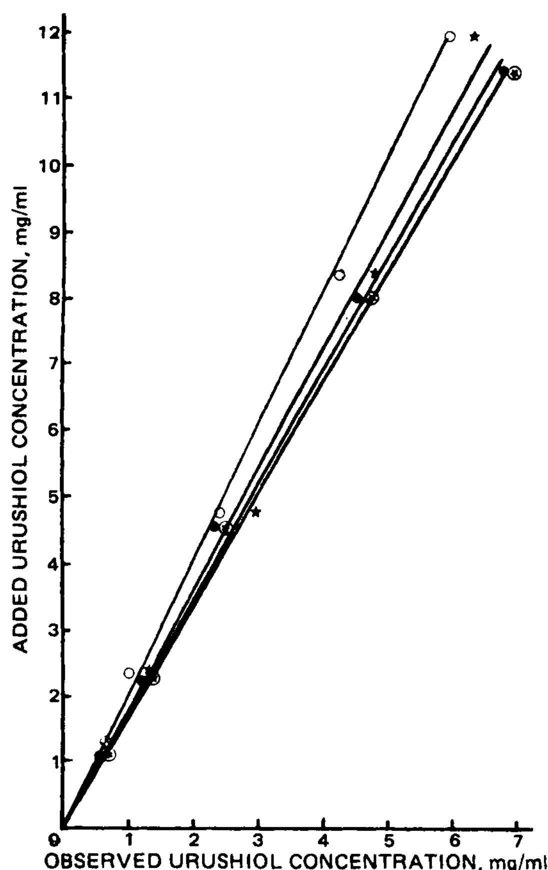

Figure 2—*Recovery curves for the extraction of urushiol from solutions of poison oak extract in corn oil (⊛), olive oil (●), seasame seed oil (★), and cottonseed oil (○) using a methanol–water (9:1) mixture.*

United States. Urushiol, the constituent in these plants that causes dermatitis, has been shown to be a mixture of 3-*n*-alk(en)yl catechols with zero, one, two, or three double bonds in the C_{15}-side chain of poison ivy (1–4) and the C_{17}-side chain of poison oak (5, 6).

Extracts of poison ivy and poison oak have been used in the detection and prophylactic treatment of sensitivity (7–9). Solutions of these extracts are provided mainly in vegetable oils, *e.g.*, corn oil (poison ivy–poison oak extract capsules[1]) and olive oil (injection[2]) (10). Although these preparations have been in use for a long time, no procedure is available to determine the concentration of the active component (urushiol) in these formulations. This report describes a simple GLC procedure for the determination of the total urushiol content of preparations containing poison ivy or poison oak extracts in vegetable oils such as corn oil, cottonseed oil, olive oil, and sesame seed oil.

EXPERIMENTAL

Plant Extracts—Poison ivy (*T. radicans*) and poison oak (*T. diversilobum*) extracts were prepared as described previously (6) or were obtained commercially[1]. The extracts were analyzed for urushiol content following the literature procedure (6).

Preparation of Oil Solutions of Poison Ivy and Poison Oak Extracts—Commercially available corn oil, cottonseed oil, olive oil, and sesame seed oil were used for the preparation of the solutions. For each oil, two sets of solutions were prepared containing 1, 2, 4, 7, or 10% of either poison ivy or poison oak extracts corresponding to 0.96–12.0 mg of urushiol/ml. The actual urushiol content of each solution (milligrams per milliliter) was based on the preanalysis of the extracts.

Extraction of Urushiol from Oil Solutions—One-milliliter aliquots of each solution were transferred to 15-ml screw-capped centrifuge tubes. To each oil solution was added 5 ml of methanol–water (9:1). The tubes were capped and vortexed for 20 sec or shaken vigorously for ~1 min and then centrifuged for 5 min. The top layer from each tube was withdrawn carefully into a 10-ml round-bottom flask, and the solvent was evaporated under vacuum at 40°.

The extraction was repeated twice using 5 ml of the same solvent each time. In each case, the top layer was added to the respective flask and the solvent was evaporated. Complete evaporation of the small amount of water remaining in the evaporation flask was achieved by adding ~2 ml each of absolute ethanol and benzene, followed by evaporation. This step was done twice to ensure complete dryness of the extract.

Preparation of Samples for GLC Analysis—*Dilute Samples*—Samples obtained from oil solutions containing <2 mg of urushiol/ml were prepared directly for GLC analysis by the addition of 0.25 ml of pyridine and 0.5 ml of bis(trimethylsilyl)trifluoroacetamide[3] containing 1% trimethylchlorosilane to the residue in the round-bottom flask. Then 0.5 ml of internal standard (dotriacontane, 5 mg/ml in isooctane) was added.

Concentrated Samples—Samples expected to contain >2 mg of urushiol/ml were treated in the following manner. The residue obtained after evaporation of the methanol–water extract was transferred quantitatively to a 5- or 10-ml volumetric flask with chloroform, and the volume was adjusted with the same solvent. A 1-ml aliquot then was withdrawn from the chloroform solution and evaporated, and the residue was treated as described for the dilute samples. The dilution factor was considered in the calculations.

GLC Analysis—The samples were analyzed using a gas–liquid chromatograph[4] and a 5% OV-225 column under the conditions reported previously (6).

[1] Supplied by Hollister-Stier Laboratories, Spokane, Wash.
[2] Ivyol injection, Merck Sharp & Dohme.
[3] Pierce Chemical Co., Rockford, Ill.
[4] Beckman GC-65.

Table I—Recovery of Urushiols from Vegetable Oil Solutions of Poison Ivy and Poison Oak Extracts Using 90% Methanol-Water [a]

	Recovery, % [b]	Correlation [b] Coefficient (r^2)	Coefficient of Variation [c], %
Corn oil			
Poison ivy	66.6	1.00	3.55
Poison oak	60.2	0.99	3.70
Olive oil			
Poison ivy	69.3	0.99	4.01
Poison oak	59.6	0.99	3.22
Sesame oil			
Poison ivy	68.4	0.99	3.50
Poison oak	52.0	0.98	3.30
Cottonseed oil			
Poison ivy	64.1	0.97	7.90
Poison oak	49.9	0.99	3.08

[a] The urushiol concentration in the different oils ranged from 0.96 to 12 mg/ml. [b] Calculated from the least-squares line. The correction factor is the reciprocal of the recovery. [c] Mean coefficient of variation of all extractions over the concentration range used.

RESULTS AND DISCUSSION

GLC procedures were described previously for the identification of urushiol (11, 12), and the use of GLC-mass spectrometry for the quantitative determination of the different congeners also was reported (13). A new GLC procedure was reported previously for the quantitation of the different components of poison ivy and poison oak urushiols as their trimethylsilyl derivatives using a 5% OV-225 column (6). The separation of the different congeners allowed the quantitation of each. The procedure was applied for the analysis of poison ivy and poison oak urushiol components in extracts obtained from different plant parts.

Extracts of poison ivy, poison oak, and, in some cases, poison sumac in vegetable oil solutions have been used for prophylactic treatment of Rhus dermatitis. The lack of an analytical procedure to determine the concentration of the active constituent, urushiol, in these preparations prompted the present study.

Corn oil, olive oil, sesame seed oil, and cottonseed oil were selected as representatives of vegetable oils. Solutions of poison ivy and poison oak extracts were made in each oil to contain different amounts of the extracts corresponding to concentrations of 0.94–12.0 mg of urushiol/ml. The extracts were analyzed for urushiol content prior to mixing.

Different cleanup procedures were investigated to separate the urushiol components from the oils prior to analysis. These procedures included column chromatography, TLC, and solvent partitioning. Due to on-column decomposition, column chromatography on silica gel 60 resulted in a loss of a significant amount of the urushiols. The loss was maximized when a large enough column was used (20 g) to permit the clean separation of the urushiol from the oil. Aluminum oxide adsorbents could not be used since urushiol binds irreversibly to alumina. Other chromatographic materials also were tried including polyamide, cellulose, cellulose acetate, and diethylaminoethylcellulose with no success.

Different solvents then were investigated for the extraction of urushiol from the oil solutions of the extracts. These solvents included methanol, ethanol, methanol-butanol mixtures (5–20% butanol), and methanol-water mixtures (2–20% water). A methanol-water mixture (9:1) was the best solvent for extraction since it gave a high percentage recovery of urushiol with the least contamination of the extracts with the oil components. GLC analysis (6) of the 90% methanol-water extracts of the pure vegetable oils showed virtually no peaks that might interfere with the urushiol peaks. Thus, the previously outlined procedure (6) was applicable to the analysis of urushiols in the methanol-water (9:1) extracts of poison ivy and poison oak preparations in vegetable oils. The extraction efficiency of this solvent mixture was determined for each oil by plotting the actual concentration of urushiol (milligrams per milliliter *versus* the observed concentration).

Figure 1 shows the recovery curves for the extraction of urushiol from solutions of poison ivy extract in different vegetable oils; Fig. 2 shows the same curves for poison oak. Each point in these curves is the average of triplicate analyses. The coefficient of variation (CV) for each point was calculated, and a mean CV value was given for each curve (Table I). A linear relationship was shown between the added and observed concentrations in each case with correlation coefficients (r^2) ranging between 0.97 and 1.00 (Table I). This result makes it possible to use this extraction procedure and to compensate for the recovery percentage. The slope of each curve (Figs. 1 and 2) thus represents the correction factor used to compensate for the extraction efficiency in each case. The same correction factors were found when preparations containing a mixture of poison ivy and poison oak extracts were formulated.

The reported procedure can be used for the analysis of the total urushiols content as well as for the percentage of each individual congener.

REFERENCES

(1) G. A. Hill, V. Mattacotti, and W. D. Grahan, *J. Am. Chem. Soc.*, **56,** 3736 (1934).
(2) W. F. Symes and C. R. Dawson, *ibid.*, **76,** 2959 (1954).
(3) S. V. Sunthankar and C. R. Dawson, *ibid.*, **76,** 5070 (1954).
(4) K. H. Markiewitz and C. R. Dawson, *J. Org. Chem.*, **30,** 1610 (1965).
(5) M. D. Corbett and S. Billets, *J. Pharm. Sci.*, **64,** 1715 (1975).
(6) J. C. Craig, C. W. Waller, S. Billets, and M. A. Elsohly, *ibid.*, **67,** 483 (1978).
(7) C. H. Ducan, *N.Y. Med. J.*, **104,** 901 (1976).
(8) G. A. Gellin, D. R. Wolf, and H. Milby, *Arch. Environ. Health,* **2,** 280 (1971).
(9) H. S. Mason and A. Lada, *J. Invest. Dermatol.*, **22,** 457 (1975).
(10) "Facts and Comparisons," Facts and Comparisons Inc., St. Louis, Mo., 1978, p. 565.
(11) H. Baer, S. Srinivasan, R. T. Bowser, and A. Karmen, *J. Allergy,* **34,** 221 (1963).
(12) S. Srinivasan, H. Baer, R. Bowser, and A. Karmen, *Anal. Biochem.*, **6,** 234 (1963).
(13) M. Gross, H. Baer, and H. M. Fales, *Phytochemistry,* **14,** 2263 (1975).

ACKNOWLEDGMENTS

Supported in part by Hollister-Stier Laboratories, Spokane, Washington, and the Research Institute of Pharmaceutical Sciences.

The authors thank Ms. Julie Crocker and Mr. William Torres for technical assistance.