Table III—Linear Regression Analysis of Average Plasma Sulfathiazole Concentration and Excretion Rate of Unchanged Sulfathiazole *versus* the Sulfathiazole Concentration in Various Tissues following Administration of Sulfathiazole, 72 mg/kg iv

Hours	Average Plasma Concentration, mg/100 ml	Average Excretion Rate, % dose/hr	Concentration in Tissues, ppm					
			Kidney	Liver	Heart	Muscle	Body Fat	Omental Fat
2.0	4.7	13.4	308	40	34	22	11	6.7
4.0	1.2	4.3	55	9.4	9.3	4.9	3.5	1.4
8.0	0.1	0.7	2.3	0.7	0.3	0.3	0.3	0.1
			r (between plasma and tissue concentrations)					
			1.000	1.000	1.000	0.999	0.998	0.998
			r (between excretion rate and tissue concentration)					
			0.990	0.996	0.998	0.995	1.000	0.992

fit" constants for averaged data shown in Table II. In each figure, the points are the averaged values from the remaining lambs at each sampling time, with 1 *SD* indicated by error bars.

Variations in the values of the rate constants due to variations within the lamb populations were estimated by fitting the data from each animal individually to the model in Scheme II. The results obtained from the averaged data were used as initial estimates. The values obtained for the parameters of Scheme II following iterative least-squares fitting to the individual animal data are presented in Table II. The average overall elimination rate constant was 0.62 ± 0.14 hr^{-1} (biological half-life of 1.1 ± 0.2 hr) and the average volume of distribution was 0.39 ± 0.04 liter/kg. These figures compare favorably with the results obtained by fitting averaged plasma and urine data and with the results obtained previously in sheep (3).

The observed one-compartment pharmacokinetics in lambs imply that the extravascular tissues into which sulfathiazole penetrates are in rapid equilibrium with plasma throughout the entire time that the drug is in the body. In the present study, the one-compartment nature of sulfathiazole pharmacokinetics beyond 2 hr can be observed in the experimentally determined drug concentrations in representative tissue specimens. This result is illustrated in Fig. 3, which shows plasma concentrations of unchanged sulfathiazole calculated using the pharmacokinetic model and concentrations of unchanged sulfathiazole in eight tissues obtained from the animals at slaughter.

According to the proposed model, the drug concentration in each tissue should be directly proportional to the plasma concentration and to the urinary excretion rate of unchanged sulfathiazole determined at the same time. The correlation coefficients obtained by linear regression of the plasma and urine data with the residual concentrations in various tissues are shown in Table III. These excellent correlations demonstrate that the plasma concentrations and the urine outputs of sulfathiazole accurately reflect tissue residues of sulfathiazole in the lamb. Thus, the time required for sulfathiazole to reach negligible levels in various tissues that might be used as food can be determined by plasma and/or urine analysis without slaughtering the animals.

REFERENCES

(1) H. D. Mercer, *Vet. Clin. N. Am.*, **5**, 3 (1975).

(2) W. G. Huber, *Pure Appl. Chem.*, **35**, 377 (1972).

(3) G. D. Koritz, D. W. A. Bourne, L. W. Dittert, and R. F. Bevill, *Am. J. Vet. Res.*, **38**, 979 (1977).

(4) R. F. Bevill, R. M. Sharma, S. H. Meachum, S. C. Wozniak, D. W. A. Bourne, and L. W. Dittert, *ibid.*, **38**, 973 (1977).

(5) J. S. Annino, in "Standard Methods of Clinical Chemistry," vol. 3, Academic, New York, N.Y., 1961, pp. 200–206.

(6) D. W. A. Bourne, R. F. Bevill, R. M. Sharma, R. P. Gural, and L. W. Dittert, *Am. J. Vet. Res.*, **38**, 967 (1977).

(7) M. Berman and M. F. Weiss, "Users Manual for SAAM," National Institute for Arthritis and Metabolic Diseases, Bethesda, Md., 1968.

ACKNOWLEDGMENTS AND ADDRESSES

Received March 8, 1976, from the *Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine, University of Illinois, Urbana, IL 61801, and the ‡College of Pharmacy, University of Kentucky, Lexington, KY 40506.

Accepted for publication December 2, 1976.

Supported by FDA Grant 74-178.

ˣ To whom inquiries should be directed.

Cascarosides A and B

J. W. FAIRBAIRN ˣ, F. J. EVANS, and J. D. PHILLIPSON

Abstract □ Electron-impact and field desorption mass spectrometry, together with NMR and circular dichroism spectroscopy, were used to confirm that cascarosides A and B are C-10 isomers of 8-*O*-(β-D-glucopyranosyl)barbaloin. Several batches of cascarosides A and B were prepared and oxidatively hydrolyzed to aloe-emodin. The results are discussed in relation to the assay for cascara given in the European Pharmacopoeia, 1971.

Keyphrases □ Cascarosides A and B—electron-impact and field desorption mass spectrometric and NMR and circular dichroism spectroscopic structural identification □ Barbaloin derivatives—cascarosides A and B, electron-impact and field desorption mass spectrometric and NMR and circular dichroism spectroscopic structural identification □ Mass spectrometry, electron impact and field desorption—structural identification of cascarosides A and B □ NMR spectroscopy—structural identification of cascarosides A and B □ Circular dichroism spectroscopy—structural identification of cascarosides A and B

Cascarosides A and B previously were isolated from *Rhamnus purshiana* DC. bark and shown to contain glucose and barbaloin (1). Later work proved that cascarosides A and B were glucosides of (+)- and (−)-barbaloin, respectively, although it was suggested that the molecules contained additional carbon fragments (2). This suggestion was partly based on the fact that the yield of barbaloin and aloe-emodin after hydrolysis was significantly less than theory for a glucoside of barbaloin. Molecular weight determinations, mass spectrometry, NMR spectroscopy, and

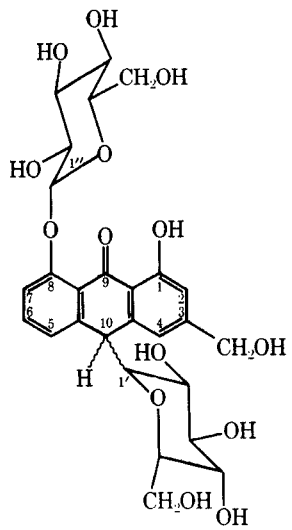
Table I—Results of Oxidative Hydrolysis of Several Independently Prepared Samples of Cascarosides

Sample Date	$E^{1\%}_{1\,cm}$ in Water at λ_{max} 267–269 nm	294–295 nm	323–325 nm	Aloe-emodin, %	Corresponding $E^{1\%}_{1\,cm}$ for Cascaroside
		Cascaroside A			
1965	119	164	140	39.8	127
1975	115	176	151	38.5	124
1976	111	173	150	40.7	131
1976	112	173	151	41.5	133
		Cascaroside B			
1961	114	161	143	40.6	130
1967	116	169	146	39.3	126
1975	118	173	149	37.2	119
1976	107	166	146	39.8	127

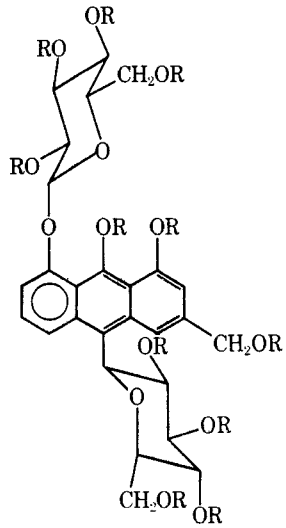
synthesis of glucosides of barbaloin were investigated, but it was not possible to confirm the presence of an additional component[1].

It was proposed (3, 4) that cascarosides A and B are 8-monoglucosides of barbaloin and that no further component is present. However, accurate molecular weight determinations were not obtained because of thermal decomposition in electron-impact mass spectrometry and because conventional methods gave values of 561 and 533 for cascarosides A and B, respectively (theoretical for dihydrate, 616). It was reported (5) that field desorption mass spectrometry clearly indicated that cascarosides A and B have a molecular weight of 580. On the basis of mass spectral and NMR studies, cascaroside A was confirmed to be the 8-*O*-β-D-glucoside of (+)-barbaloin and cascaroside B was reported to be the corresponding diastereoisomer of (−)-barbaloin (5). Independently, Wagner and Demuth (6) reached the same conclusion on the basis of partial hydrolyses, sugar analyses, and spectral data.

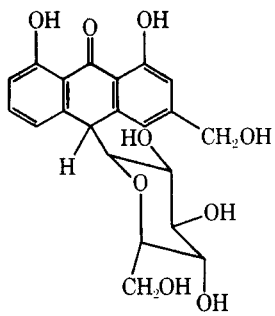
The present report gives details of spectral data that confirm and amplify those already published. In addition, several new batches of cascarosides were prepared to confirm whether the yield of aloe-emodin previously reported is correct. This value has been used as the basis of an officially recommended colorimetric method for the assay of cascara bark and extract (7) and is now incorporated in the European Pharmacopoeia, 1971.



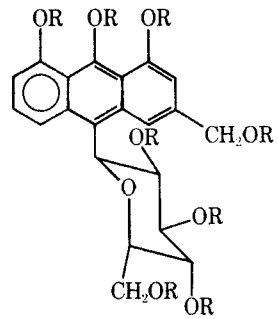
I



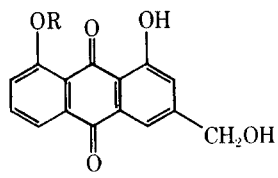
IIa: R = CH_3CO
IIb: R = $SiH(CH_3)_3$



III



IVa: R = CH_3CO
IVb: R = $SiH(CH_3)_3$



Va: R = glucose
Vb: R = H

EXPERIMENTAL[2]

Isolation of Cascarosides A and B—The method used was previously described (1), except that separation of cascarosides A and B was effected by paper chromatography rather than by countercurrent methods. The bands corresponding to cascarosides A and B on 3-mm paper[3], after development with ethyl acetate–methanol–water (100:17:13) (8), were eluted with methanol, and the eluates were evaporated to dryness. The residues were extracted with hot 2-propanol and filtered, and the filtrates were allowed to stand in the dark at room temperature overnight. The microcrystalline precipitates that formed were collected and dried.

Further yields were obtained by evaporating the supernate and dissolving the residue in 10 volumes of anhydrous methanol (filtering off insoluble matter if necessary). Ethyl methyl ketone was added slowly with vigorous stirring until the solution became cloudy. After centrifuging, a reddish-yellow precipitate formed, which was discarded.

The process was repeated until the precipitate that formed was bright yellow. At this stage, ethyl methyl ketone–methanol (10:1) was added. The bright-yellow precipitate was allowed to settle and was then filtered off. Before becoming dry, it was washed with methanol–petroleum ether (bp 40–60°) (1:1) and then petroleum ether and dried *in vacuo*.

Cascaroside A (I)—UV data are presented in Table I. Paper chromatography R_f values were as previously reported (1). Electron-impact mass spectrum: no $M^{+\cdot}$, *m/e* (relative intensity) 256 (100%), further fragmentation similar to that of barbaloin. Field desorption mass spectrum, 17 mamp and 80°, from methanol: *m/e* 603 ($M^{+\cdot}$ + [Na^+], 100%), 620 ($M^{+\cdot}$ + [K^+], 5), 605 ($M^{+\cdot}$ + [Na^+ + 2], 8), 604 ($M^{+\cdot}$ + [Na^+ + 1], 18), 602 (M^+ + [Na^+ − 1], 40), and 580 ($M^{+\cdot}$, 12); from water: *m/e* 674 ($M^{+\cdot}$ + [(Na^+ × 4) + 2], 12%), 620 (40), 606 (M^+ + [Na^+ + 3], 14), 605 (12), 604 (8), 603 (100), 602 (9), and 580 (10). Fragment ions consistently present in spectra obtained from methanol or from water: *m/e* 442 ($M^{+\cdot}$ + [Na^+ − 161], 50%), 418 ($M^{+\cdot}$ − [162], 12), 280 (100), and 256 (20). NMR (dimethyl sulfoxide-d_6): δ 3.0–3.50 (glucosyl hydrogens), 4.68 (3H, s, aryl CH_2O and aryl-CH–aryl), 5.10 (1H, m, H-1′), 6.84 (1H, broad s, H-2), 7.0 (1H, broad s, H-4), 7.1–7.80 (3H, m, H-5, H-6, and H-7), and 11.89 (1H, s, aryl OH) ppm. Circular dichroism spectrum (methanol): 4.39 mg/5 ml, Δε +4.6 (355 nm), −8.8 (316), −2.8 sh (282), +1.4 sh (258), +4.4 sh (240), +5.0 (234), and −13.2 (211).

Cascaroside B (I)—UV data are presented in Table I. Paper chromatography R_f values were as previously reported (1). Electron-impact mass spectrum: no $M^{+\cdot}$, *m/e* (relative intensity) 256 (100%), further fragmentation similar to that of barbaloin. Field desorption mass spec-

[1] Unpublished observations.

[2] Mass spectra were obtained on an AEI MS 902 mass spectrometer at 70 ev by direct insertion at 180°. NMR spectra were obtained using a Perkin-Elmer 12A 60-MHz spectrometer with trimethylsilane = 0.00 ppm. Cascara bark of European Pharmacopoeia (1971) standard was obtained from W. Ransom and Son Ltd., Hitchin, England.

[3] Whatman.

trum, 17 mamp and 80°, from methanol: *m/e* 604 (18%), 603 (70), 581 (23), 580 (M^{+}·, 44), 280 (100), and 256 (100); from water: *m/e* 605 (8%), 604 (8), 603 (100), 580 (18), 442 (80), 280 (75), and 256 (68). NMR (dimethyl sulfoxide-d_6): δ 3.0–3.8 (glucosyl hydrogens), 4.54 (3H, s, aryl CH_2O and aryl–CH–aryl), 4.90 (1H, m, H-1′), 6.64–7.6 (5H, m, H-2, H-4, H-5, H-6, and H-7), and 12.1 (1H, s, aryl OH) ppm. Circular dichroism spectrum (methanol): 2.63 mg/ml, $\Delta\epsilon$ −1.8 (368 nm), +1.0 (324), −1.2 (290), −0.8 (263), +0.3 (251), −7.0 (230), and +9.2 (210).

Cascaroside Acetate (IIa)—Cascarosides A and B (20 mg of each) were dissolved separately in 4 ml of anhydrous pyridine–acetic anhydride (2:1) and allowed to stand in the dark and under nitrogen for 2 days. Excess water was added, and the resulting suspension was centrifuged. The residues were washed with water, recentrifuged until free from pyridine, and dried *in vacuo* at 60° for 24 hr. TLC [silica gel GF, 1-propanol–ethyl acetate–water (40:30:40)] indicated that the products from cascarosides A and B were identical.

Electron-impact mass spectrum: no M^{+}·, *m/e* (relative intensity) 653 (M^{+}· − [acetylglucose], 17%), 494 (2), 452 (7), 410 (14), 368 (21), and 331 (100). Field desorption mass spectrum, 14 mamp, from chloroform: *m/e* 1042 (M^{+}·, 100%) and 1043 (M^{+}· + [1], 40). NMR (deuterochloroform): δ 1.82–2.53 (11 × 3H, s, 11 × CH_3CO), 5.33 (2H, s, aryl CH_2O), 5.95 (1H, broad s, H-1′), and 7.0–8.93 (5H, m, H-2, H-4, H-5, H-6, and H-7) ppm.

Cascaroside Trimethylsilyl Ether (IIb, R = Trimethylsilyl)—Cascarosides A and B (20 mg of each) were dissolved separately in 2 ml of anhydrous pyridine. To each solution were added 0.5 ml of hexamethyldisilazane and 0.5 ml of trimethylchlorosilane. After standing for 5 min at room temperature, the solvents were removed by vacuum distillation. The dry residue was extracted with carbon tetrachloride and filtered, and the solutions were evaporated to 0.5 ml for NMR spectroscopy.

The NMR spectra obtained from the trimethylsilyl ethers of cascarosides A and B were identical: δ 3.50 (12H, m, glucosyl hydrogens), 4.35 (1H, m, H-1′), 5.15 (1H, m, H-1″), 4.66 (2H, s, aryl CH_2O), and 6.9–7.5 (5H, m, H-2, H-4, H-5, H-6, and H-7) ppm.

Barbaloin (III)—This compound was prepared from commercial aloin (9). Electron-impact mass spectrum: *m/e* (relative intensity) 418 (M^{+}·, 0.5%), 256 (M^{+}· − [162], 100), 238 (M^{+}· − [162 + 18], 5), 228 (M^{+}· − [162 + 28], 7), 210 (M^{+}· − [162 + 28 + 18], 4), 197 (M^{+}· − [162 + 28 + 31], 3), 181 (5), and 165 (3). Field desorption mass spectrum, 14 mamp, from methanol: *m/e* 418 (M^{+}·, 100%) and 256 (M^{+}· − [162], 11). NMR (dimethyl sulfoxide-d_6): δ 2.78–3.50 (6H, m, glucosyl hydrogens), 4.58 (3H, 3, aryl CH_2O and aryl–CH–aryl), 5.1 (1H, m, H-1′), 7.60 (1H, t, H-6), 7.05 (1H, d, H-5), 6.90 (1H, d, H-7), 7.05 (1H, s, H-4), 6.88 (1H, s, H-2), and 11.3 and 11.4 (2 × 1H, s, aryl OH) ppm.

Barbaloin Acetate (IVa)—Aloin (5 mg) was converted into the acetate by the method described under *Cascaroside Acetate*. TLC [silica gel G, 1-propanol–ethyl acetate–water (40:30:40)] showed the presence of a single compound. Electron-impact mass spectrum: *m/e* (relative intensity) 754 (M^{+}·, 5%), 713 (<5), 694 (<5), 676 (30), 652 (40), 628 (30), 611 (10), 568 (12), 550 (6), 508 (11), 448 (25), and 331 (100). Field desorption mass spectrum, 17 mamp, from methanol: *m/e* 754 (M^{+}·, 100%), 755 (50), 756 (20), 714 (10), and 715 (10). NMR (carbon tetrachloride): δ 1.91–2.40 (8 × 3H, s, 8 × CH_3CO), 5.40 (2H, s, aryl CH_2O), 6.00 (1H, broad s, H-1′), and 7.0–8.95 (5H, m, H-2, H-4, H-5, H-6, and H-7) ppm.

Barbaloin Trimethylsilyl Ether (IVb)—Aloin (20 mg) was converted into its trimethylsilyl ether by the method described for IIb. NMR (carbon tetrachloride): δ 3.23 (6H, m, glucosyl protons), 4.18 (1H, m, H-1′), 4.43 (2H, s, aryl CH_2O), 6.60 (4H, m, H-2, H-4, H-5, and H-7), and 7.05 (1H, m, H-6) ppm.

Aloe-emodin 8-Monoglucoside[4] (Va)—Electron-impact mass spectrum: no M^{+}·, *m/e* (relative intensity) 270 (M^{+}· − [glucose], 100%), 253 (M^{+}· − [17], 6), 241 (M^{+}· − [29], 58), 225 (M^{+}· − [17 + 28], 12), 213 (M^{+}· − [29 + 28], 17), 197 (M^{+}· − [17 + 56], 7), 185 (M^{+}· − [29 + 56], 5), 139 (24), and 121 (33). Field desorption mass spectrum, 17 mamp, from methanol: *m/e* 432 (M^{+}·, 100%), 433 (M^{+}· + [1], 65), 455 (M^{+}· + [Na^{+}], 100), 269 (90), 271 (70), and 270 (75). NMR (dimethyl sulfoxide-d_6): δ 3.40 (6H, m, glucosyl hydrogens), 4.55 (2H, s, aryl CH_2O), 7.25–7.90 (5H, m, H-2, H-4, H-5, H-6, and H-7), and 10.91 (1H, s, aryl OH) ppm.

Aloe-emodin (Vb)—This compound was prepared from barbaloin by oxidative hydrolysis using the method described previously (9). Electron-impact mass spectrum (280°): *m/e* (relative intensity) 270 (M^{+}·, 100%), 253 (M^{+}· − [17], 4), 242 (M^{+}· − [28], 4), 225 (M^{+}· − [17 + 28], 5), 224 (M^{+}· − [18 + 28], 6), 213 (M^{+}· − [29 + 28], 8), 197 (M^{+}· − [17 + 56], 3), 185 (2), 139 (8), and 121 (12). Metastable peaks corresponding to the following transitions were observed, $270 \xrightarrow{237.1} 253$, $270 \xrightarrow{216.9} 242$, $242 \xrightarrow{187.5} 213$, and $242 \xrightarrow{207.3} 224$. NMR (dimethyl sulfoxide-d_6): δ 4.62 (2H, s, aryl CH_2O), 7.33 (2H, m, H-2 and H-7), and 7.73 (3H, m, H-4, H-5, and H-6) ppm.

Oxidative Hydrolysis of Cascarosides A and B to Aloe-emodin—Four separate batches of cascaroside A and four of cascaroside B were prepared as described. Their purity was checked by paper chromatography, UV spectrum, melting point, and, mainly, the $E^{1\%}_{1\,cm}$ values at the UV_{max} (these varied less than the melting point) (1).

Quantities of 10–20 mg were dried *in vacuo* over magnesium chlorate at 90–100° for 1 hr to remove moisture and water of crystallization. The anhydrous material was dissolved in 50 ml of water, and 10 ml was subjected to ferric chloride–hydrochloric acid treatment (10). The aloe-emodin produced was measured colorimetrically in 1 *N* NaOH at 500 nm, and the quantity was calculated using the $E^{1\%}_{1\,cm}$ value of 320. The corresponding $E^{1\%}_{1\,cm}$ value for the cascarosides was also calculated (Table I).

DISCUSSION

Spectrometry—Electron-impact mass spectrometry can be used for the characterization of the aglycone aloe-emodin (Vb) since the M^{+}· ion was observed as the base peak at *m/e* 270 and further fragmentation was observed by ion peaks at *m/e* 253, 242, 225, 224, and 213. However, electron-impact mass spectrometry of the *O*-glycoside aloe-emodin 8-glucoside (Va) resulted in the absence of an M^{+}· ion and a spectrum that was similar to that of the aglycone. Electron-impact mass spectrometry of the C-glycoside barbaloin (III) showed only a small M^{+}· ion at *m/e* 418 (0.5%), and the base peak was observed at *m/e* 256. Further fragmentation occurred by loss of 16, 28, or 29 mass units progressively from the M^{+}· ion, similarly to that of aloe-emodin and other anthraquinones.

Furthermore, electron-impact mass spectrometry did not result in the presence of an M^{+}· ion for either cascaroside A or B, the highest mass ion fragment again occurring at *m/e* 256 due to the loss of both sugar units from the molecule. Electron-impact mass spectrometry of the acetate, which is common to cascarosides A and B, also failed to produce an M^{+}· ion, the highest mass fragment ion being observed as a peak at *m/e* 653, corresponding to the loss of acetylglucose.

Ever since the cascarosides were first isolated (1), attempts at characterization have failed largely because of the difficulty in obtaining accurate molecular weights (2, 4). A solution to this problem is field desorption mass spectrometry (5, 6). Spectra of cascarosides A and B were obtained from aqueous and methanolic solutions, and the presence of quasimolecular ions such as M^{+}· + [Na^{+}], M^{+}· + [K^{+}], and M^{+}· + [H^{+}] were of use in establishing that the M^{+}· ion occurs at *m/e* 580. Peaks corresponding to the presence of such ions in the spectra of glycosides from polar solvents also were reported for flavonoids (11), and their significance to molecular weight determination was discussed (12). To eliminate the formation of quasimolecular ions, the acetates of both cascarosides were prepared (IIa). The M^{+}· ion occurred at *m/e* 1042 (100%), thus confirming the molecular weight of the cascarosides as 580. This result is also in agreement with the suggestions (2) that three acetyl moieties are present in the aglycone position and that the tetrahedral arrangement at C-10 is lost on acetylation.

Field desorption mass spectrometry was superior to electron-impact mass spectrometry for the molecular weight determination of other anthraquinone glycosides since the spectra of barbaloin (III) and of its acetate (IVa) had base peaks at *m/e* 418 and 754, respectively, corresponding to the M^{+}· ions. The spectrum of aloe-emodin 8-glucoside (Va) also showed the presence of an M^{+}· ion peak, which occurred at *m/e* 432 (100%); quasimolecular ions were formed as illustrated by the presence of a peak at *m/e* 455, corresponding to M^{+}· + [Na^{+}].

The NMR spectra of cascarosides A and B and of the related compounds were also important in confirming the structure of cascarosides A and B. Signals for the five aromatic hydrogens between δ 7.33 and 7.73 ppm and for the aromatic methylenehydroxy at δ 4.62 ppm were readily observed in the NMR spectrum of the aglycone aloe-emodin. For Va, a six-proton glucosyl envelope was observed at δ 3.4 ppm in addition to the signals of the five aromatic hydrogens, the aromatic methylenehydroxy, and the phenolic hydrogen, which appeared at δ 10.91 ppm.

In the NMR spectrum of barbaloin (III), a six-proton glucosyl envelope at δ 2.78–3.50 ppm was also evident, and the presence of a three-proton singlet at δ 4.58 ppm was attributed to the aromatic methylenehydroxy and H-10 protons. The H-1′ signal appeared at δ 5.1 ppm, and the five aromatic protons showed as a multiplet between δ 6.88 and 7.60 ppm; two

[4] Supplied by Professor H. Wagner.

downfield singlets at δ 11.3 and 11.4 ppm were due to the hydrogen-bonded phenolic hydrogens. Signals for eight acetyl groups were observed in the spectrum of IV*a* between δ 1.90 and 2.40 ppm, and the signal for the hydrogen at C-10 disappeared. A similar result was also obtained for the NMR spectrum of IV*b*.

In the NMR spectrum of cascaroside A, the H-1′ glucosyl proton appeared as a singlet at δ 5.10 ppm, and the methylenehydroxy and H-10 proton appeared together as a 3H singlet at δ 4.68 ppm. Signals for five aromatic protons between δ 6.84 and 7.80 ppm and a single hydrogen-bonded phenolic proton signal at δ 11.89 ppm were also evident. However, because of the difficulty in removing traces of moisture from the cascaroside, it was not possible to assess the number of protons in the glucosyl envelope between δ 3.0 and 3.50 ppm. A similar NMR spectrum was obtained for cascaroside B.

Therefore, derivatives of cascarosides A and B were produced to ascertain the number of glucosyl protons. The acetate (II*a*) common to cascarosides A and B showed the presence of 11 acetyl group signals at δ 1.82–2.53 ppm and the absence of the H-10 signal in an analogous way to the spectrum of barbaloin acetate. NMR of II*b* showed a 12H multiplet at δ 3.50 ppm, representing the proton envelope of the two glucose residues. The other two glucosyl protons at H-1′ and H-1″ produced signals at δ 4.35 and 5.15 ppm, respectively.

Hence, the application of field desorption mass and NMR spectral techniques to cascarosides A and B, the aglycone-aloe-emodin, and its monoglucosides barbaloin and aloe-emodin 8-glucoside confirms that the cascarosides are *O*-glucosides of barbaloin and that, despite previous hydrolysis results (2), no additional moieties are present. Partial hydrolysis of cascarosides A and B indicated that the *O*-glucose is situated at C-8 and enzyme hydrolysis confirmed that this linkage is beta (6). Partial hydrolysis of cascarosides A and B resulting in (+)- and (−)-aloins, respectively (2), confirmed that cascaroside A is 8-*O*-β-D-glucopyranosyl-(+)-barbaloin and that cascaroside B is 8-*O*-β-D-glucopyranosyl-(−)-barbaloin, in agreement with the proposals of Wagner and Demuth (6). The circular dichroism spectra (see *Experimental*) of cascarosides A and B clearly showed that the two compounds are diastereoisomers that can be represented as I.

Oxidative Hydrolysis—The results in Table I confirm earlier findings (10) that anhydrous cascarosides A and B only yield about 40% aloe-emodin (theoretical 46.6%). Since the molecular weight of these cascarosides is now established to be 580, if they are quantitatively converted into aloe-emodin (molecular weight 270, $E^{1\%}_{1\,cm}$ = 500 nm in 1 *N* NaOH gives 320), they should have a corresponding $E^{1\%}_{1\,cm}$ value of 149. The average value in Table I is 127, which is very close to the value of 125 based on earlier samples and used in the recommended method (7). Therefore, the equivalent amount of dihydroxyanthraquinone used in the European Pharmacopoeia, 1971, should stand.

The comparatively low yield of aloe-emodin (86% of theory) is in marked contrast to results with barbaloin in identical oxidative hydrolysis conditions when almost quantitative conversion to aloe-emodin takes place (63.6% found, 64.6% theory) (9). Although Wagner and Demuth (6) obtained almost theoretical yields from samples of cascarosides A and B, this result was based on comparative oxidative hydrolysis of cascarosides and aloin. In absolute terms, their experimental results indicated that their cascaroside A yielded 21.3% aloe-emodin, their cascaroside B yielded 21.5% aloe-emodin, and their barbaloin yielded 31% aloe-emodin (theoretical 64.6%). Clearly, their oxidative hydrolysis conditions did not completely convert to aloe-emodin. Furthermore, their extinctions of the final red solution were not read at the maximum of about 500 nm ($E^{1\%}_{1\,cm}$ = 320) but at 546 nm. From our own curves of aloe-emodin in 1 *N* NaOH, the $E^{1\%}_{1\,cm}$ value at this wavelength is 194; this latter value was used in making our calculations.

REFERENCES

(1) J. W. Fairbairn and S. Simic, *J. Pharm. Pharmacol., Suppl.*, **12,** 45T (1960).

(2) J. W. Fairbairn, C. A. Friedmann, and S. Simic, *ibid.*, **15,** 292T (1963).

(3) G. Demuth, Inaugural Dissertation, L.-Maximil. Universität, München, West Germany, 1972.

(4) H. Wagner and G. Demuth, *Z. Naturforsch.*, **29c,** 204 (1974).

(5) F. J. Evans, J. W. Fairbairn, and J. D. Phillipson, *J. Pharm. Pharmacol., Suppl.*, **27,** 91P (1975).

(6) H. Wagner and G. Demuth, *Z. Naturforsch.*, **31b,** 267 (1976).

(7) *Analyst*, **98,** 830 (1973).

(8) L. Hörhammer, H. Wagner, and G. Bittner, *Pharm. Ztg.*, **108,** 259 (1963).

(9) J. W. Fairbairn and S. Simic, *J. Pharm. Pharmacol.*, **15,** 325 (1963).

(10) *Ibid.*, **16,** 450 (1964).

(11) H. R. Schulten and H. D. Beckey, *Org. Mass Spectrom.*, **7,** 1103 (1973).

(12) H. D. Beckey and H. R. Schulten, *Angew. Chem. Int. Ed.*, 14 (6), 403 (1975).

ACKNOWLEDGMENTS AND ADDRESSES

Received August 2, 1976, from the *Department of Pharmacognosy, School of Pharmacy, University of London, London WC1N 1AX, England.*

Accepted for publication December 1, 1976.

The authors are indebted to Dr. D. E. Games, Department of Chemistry, University of Wales, Cardiff, for determining the field desorption mass spectra, to Dr. P. M. Scopes, Department of Chemistry, Westfield College, University of London, for determining the circular dichroism spectra, and to Mr. D. Carter and Mr. W. Baldeo, Department of Pharmaceutical Chemistry, School of Pharmacy, University of London, for determining the mass and NMR spectra, respectively. They also thank Professor H. Wagner, University of Munich, West Germany, for a sample of aloe-emodin 8-glucoside. They are grateful to Mr. M. Bragg for technical assistance.

[x] To whom inquiries should be directed.