TLC showed the presence of a compound in the enzyme reaction mixture and the ether extract which was neither lactate nor pyruvate nor dimethyl sulfoxide. The R_f value for this compound was identical with that for 2-hydroxy-2-methyl-3-methylsulfinylpropanoic acid. No intermediate spot could be detected in the absence of dimethyl sulfoxide in the reaction medium.

DISCUSSION

Baker *et al.* (4) showed that the lactate dehydrogenase reaction sites are the same. Therefore, inhibition at the reaction site should decrease the rates of both the forward and the reverse reactions. The increase in the forward reaction rate and the decrease in the rate of the reverse reaction could be explained by the presence of a concentration gradient on the reversible reaction in a direction favoring pyruvate formation. Such a concentration gradient could result from a complexation or condensation of dimethyl sulfoxide with pyruvic acid, thus removing that species from the reaction medium. A possible reaction that could remove effectively the keto acid salt (pyruvate) is an aldol-type condensation between the methyl groups of dimethyl sulfoxide and the carbonyl function of the pyruvate. Such a condensation may involve either a single methyl group or both methyl groups of the dimethyl sulfoxide (Scheme II). The effect on the enzyme-catalyzed reaction would be concentration dependent (Tables I and II).

The presence of 2-hydroxy-2-methyl-3-methylsulfinylpropanoic acid in the enzyme mixture leads to the conclusion that dimethyl sulfoxide interferes with the lactate dehydrogenase-catalyzed reaction by condensing with pyruvate in an aldol-type condensation.

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ACKNOWLEDGMENTS AND ADDRESSES

Received August 23, 1971, from the *College of Pharmacy, University of South Carolina, Columbia, SC 29208*

Accepted for publication March 8, 1973.

Abstracted in part from a thesis submitted by R. E. Ledesma to the College of Pharmacy, University of South Carolina, in partial fulfillment of the Master of Science degree requirements.

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Cinchona Alkaloids on Ion-Exchange Resins V: Ammonium Form of Resins

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Abstract □ Equilibrium exchange of cinchona alkaloid sulfates and column runs of cinchona bark were carried out with the ammonium form of sulfonic acid cation-exchange resins. The results indicate that, for 100 meq. of the resin capacity, the amounts of the alkaloid-containing material extracted from cinchona bark are 15 and 8 g. for the ammonium forms of two commercially available ion-exchange resins. Dilute aqueous sulfuric acid was used as the extraction solvent, and ammonical ethanol was used as the eluant.

Keyphrases □ Cinchona alkaloids—separation from cinchona bark using ion-exchange resins in ammonium form, comparison of two commercially available resins, separation capacity □ Alkaloids, cinchona—separation from cinchona bark using ion-exchange resins in ammonium form, comparison of two commercially available resins, separation capacity □ Resins, ion exchange, in ammonium forms—comparison of separation capacities of two commercially available resins, cinchona alkaloids □ Ion-exchange chromatography—comparison of separation capacities of two commercially available resins, cinchona alkaloids □ Chromatography, ion exchange—comparison of separation capacities of two commercially available resins, cinchona alkaloids

A previous paper (1) reported the effect of the particle size of styrene divinylbenzene copolymer-based sulfonic acid cation-exchange resins on equilibrium exchange and column studies for cinchona alkaloid sulfates. The work suggested that a fine mesh resin with a relatively low degree of cross-linking (Resin I[1]) or with an expanded structure (Resin II[2]) in ammonium form could be suitable for recovery of cinchona alkaloids, with aqueous sulfuric acid as the solvent for the exchange run and ammoniacal ethanol as the solvent for the elution run. The present work includes a study of equilibrium exchange of cinchona alkaloid sulfates and column runs of cinchona bark with the ammonium form of sulfonic acid cation-exchange resins.

EXPERIMENTAL

Resins—The resins were conditioned and converted into hydrogen form. The experimentally estimated values of percent moisture content and capacity in milliequivalents per gram of air-dried resin were: Resin I (50–100), 24.0, 3.89; Resin I (100–200), 22.0, 3.72; and Resin II (20–50), 26.0, 3.53.

The ammonium form of the resin was prepared by treating the hydrogen form with excess aqueous ammonia. The moisture content of the air-dried resin in the ammonium form was estimated, and its capacity was calculated by taking the oven-dried capacity of the resin in hydrogen and ammonium forms to be the same.

Chemicals and Materials—Chemicals were from earlier stocks. Cinchona bark[3] was powdered below 60 mesh, and samples were estimated for alkaloid content (2). The value was 6.5%. Samples of extracted alkaloid material were analyzed (3), and the mixture of quinine and cinchonidine obtained was estimated for each by UV absorption (4). The percent analysis was: quinine, 18.0; quini-

[1] Dowex 50W-X4 (50–100, 100–200).

[2] Amberlite-200 (20–50).

[3] Obtained from the Government Quinine Factory, West Bengal, India.

Table I—Equilibrium Exchange[a] of Aqueous Quinine Sulfate and Cinchonidine Sulfate with Cation-Exchange Resins in Ammonium Form

Resin	Quinine Sulfate			Cinchonidine Sulfate		
	$[\bar{A}]_e$	P_A	P_R	$[\bar{A}]_e$	P_A	P_R
Resin I (50–100)	0.49	20.5	49.0	0.45	21.0	68.1
	0.79	33.5	50.1	0.80	37.5	68.8
	1.30	55.0	51.4	1.26	59.0	69.3
	1.74	73.5	52.1	1.62	76.0	69.6
Resin II (20–50)	0.63	28.5	26.3	0.52	25.0	29.0
	0.89	40.5	25.3	0.76	37.0	27.4
	1.29	58.5	23.9	1.10	53.5	25.1
	1.76	80.0	22.6	1.56	76.0	22.3

[a] $[\bar{A}]_e$ = Milliequivalents of alkaloid in the resin phase per liter of solution, at equilibrium; P_A = percent exchange of alkaloid sulfate at equilibrium; and P_R = percent resin capacity exchanged at equilibrium. Effective capacity = (capacity of the resin) (average value of P_R).

dine, 17.5; cinchonine, 29.0; cinchonidine, 9.0; and the rest (by difference), 26.5.

Procedure—The procedure for equilibrium exchange of cinchona alkaloid (quinine, quinidine, cinchonine, and cinchonidine) sulfates with ammonium forms of Resin I (50–100) and Resin II (20–50) was same as given earlier (5, 6).

Columns, each containing 100 meq. of the resin in the ammonium form, were set up (1).

The values of bed length (cm.) and bed volume (ml.) were: for Resin I (100–200), 44.1, 75.0; and for Resin II (20–50), 35.0, 59.5.

The procedure for carrying out a run was as follows. A weighed amount of the powdered cinchona bark was stirred with 0.01 *N* aqueous sulfuric acid solution (4 l.), allowed to stand overnight, and filtered. The filtrate was transferred to an overhead aspirator and passed through the column at a flow rate of 10 ml./min. The effluent was collected, and its acid strength was adjusted to about 0.01 *N* by pH measurement; then it was returned to the container of bark, stirred well, and filtered. The filtrate was transferred to the overhead aspirator and thus the run was continued. The run was discontinued when the alkaloids were almost completely extracted from cinchona bark, as indicated by absence of fluorescence, or when the alkaloid content of the effluent and influent was almost same, as indicated by fluorescence when exposed to UV radiation. Then the column was washed with distilled water and backwashed to remove extraneous materials from the resin bed. Fifty percent aqueous ethanol (50 ml.) was passed through the column.

The alkaloid material was eluted with 0.1 *N* ammoniacal ethanol at a flow rate of 1.5 ml./min. The effluent containing alkaloids was concentrated by distillation, evaporated on a water bath, dried at 100 ± 1°, weighed, and analyzed.

The resin in the column was then replaced by an equivalent amount of resin from stock, backwashed, and allowed to settle under gravity. The column then was ready for the next run.

RESULTS AND CONCLUSIONS

Equilibrium Exchange Study—The equilibrium exchange study was carried out for the cinchona alkaloid (quinine, quinidine, cinchonine, and cinchonidine) sulfates with Resin I (50–100) and Resin II (20–50) in the ammonium form. Table I gives the results for quinine sulfate and cinchonidine sulfate. From the results obtained, it may be concluded that:

Table II—Data for Column Runs with Cinchona Bark

	Resin I	Resin II
Weight, g.[a]	1000	357
Amount of acid[b] solution passed, l.	44	24
Amount of eluant[c] used, l.	6	3
Weight of extracted material, g.	15	8

[a] Weight (grams) = weight (grams) of cinchona bark taken for the run. [b] Acid = 0.01 *N* sulfuric acid. [c] Eluant = 0.1 *N* ammoniacal ethanol.

1. The value of P_R (the percent resin capacity exchanged at equilibrium) for the optical isomers (quinine and quinidine or cinchonine and cinchonidine) is not measurably different.
2. The value of P_R for quinine sulfate is lower than that of cinchonidine sulfate.
3. The value of P_R for Resin II decreases as the value of P_A (the percent exchange of alkaloid sulfate at equilibrium) increases.

These results are similar to those obtained earlier (5, 6).

Column Study—Runs were carried out starting with different amounts of powdered cinchona bark. From the results obtained, it is concluded that:

1. When the alkaloid content (milliequivalents) of the powdered cinchona bark is lower than the capacity (milliequivalents) of the resin in the column, the alkaloid material present in the bark is extracted.
2. When the alkaloid content of the powdered cinchona bark is much greater than the capacity of the resin in the column, the amount of extracted alkaloid-containing material is 15 g. for Resin I and 8 g. for Resin II (capacity of the resin in the column being 100 meq.). Thus, the amount of the alkaloid-containing material obtained is close to the effective capacity of the column (7). Table II gives the data for runs with excess powdered cinchona bark.

The results indicate that for the extraction of alkaloids from cinchona bark with the ammonium form of the resin, dilute aqueous sulfuric acid may be used as the extraction solvent and ammoniacal ethanol may be used as the eluent. It would be desirable to assess the stability of the resins for repeated use over time.

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ACKNOWLEDGMENTS AND ADDRESSES

Received July 13, 1972, from the *Chemistry Department, M.S. University of Baroda, Baroda-2, India.*

Accepted for publication March 1, 1973.

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