Identification of Polymeric 5,7,3′,4′-Tetrahydroxyflavan-3,4-diol from Tannin Extract of Powdered Cassia Bark, Saigon, Cinnamon USP, *Cinnamomum loureirii* Nees, Family Lauraceae

LEONARD BUCHALTER†

Abstract □ A phytochemical investigation of a tannin extract from powdered cinnamon USP showed the presence of flavanoidal-type condensed tannins. The tannin extract consisted of polymeric 5,7,3′,4′-tetrahydroxyflavan-3,4-diol units. No monomeric leucoanthocyanidins or polyphenolic derivatives of benzoic or cinnamic acid were present in the tannin extract. Identification was accomplished by paper chromatography and visible and IR spectrophotometry.

Keyphrases □ 5,7,3′,4′-Tetrahydroxyflavan-3,4-diol (polymeric)—identification □ Tannin extract—identification of polymeric 5,7,3′,4′-tetrahydroxyflavan-3,4-diol □ Paper chromatography—identification □ IR and visible spectrophotometry—identification

The presence of tannins in several plant extracts has been reported but, in many cases, an exact phytochemical investigation of the tannins was not carried out. In previous papers (1, 2), the author phytochemically evaluated tannin fractions from the tubers of *Rumex hymenosepalus*, Family Polygonaceae, and wild cherry bark USP, *Prunus serotina* Erhart, Family Rosaceae. Tannin content has been reported for the powdered cassia bark of *Cinnamomum loureirii* Nees, Family Lauraceae (3). A phytochemical investigation of this material was undertaken as part of a continuing investigation into the chemical nature of the reported tannin content of drug extracts.

EXPERIMENTAL

Materials—Paper chromatography was carried out on Whatman No. 1 filter paper in glass tanks. IR spectra were made on a Beckman IR 8 spectrophotometer using a KBr pellet. Visible spectra were obtained from a Beckman DB spectrophotometer. Commercial samples of cyanidin[1] and powdered cinnamon bark USP[2] were used.

Preparation of Tannin Extract—Five hundred grams of powdered bark of cinnamon USP, *Cinnamomum loureirii* Nees, was defatted with successive washings of petroleum ether and chloroform. The marc was extracted with 500 ml. of water–ethanol (1:1) and then filtered. The resulting brown solution was evaporated *in vacuo*, yielding a dark-brown amorphous residue. This material was tested for tannin content with 5% alcoholic ferric chloride solution. Gelatin and alkaloidal precipitation tests were also used. A deep-green color was obtained with the ferric chloride reagent, and the gelatin and alkaloidal precipitation tests proved positive for tannins.

Hydrolysis Tests—A portion of the extract was subjected to hydrolytic procedures in both acid and alkaline media. The extract was found to be of nonhydrolyzable tannins.

Paper Chromatography—A portion of the tannin extract, dissolved in a 1:1 mixture of ethanol–water, was spotted on Whatman No. 1 filter paper and exposed to paper chromatography in the following solvent systems: Solvent 1, butanol–acetic acid–water (4:1:5), organic phase; Solvent 2, butanol–acetic acid–water (4:1:5), aqueous phase; and Solvent 3, 10% acetic acid. In all solvent systems, only a long very light-brown streak running from the origin to very high R_f values was apparent. No single, visible, or UV fluorescent materials were apparent on the chromatograms. All three papers, when sprayed with 5% alcoholic ferric chloride and heated in an oven, showed only a solid gray-green streak from the origin to high R_f values. All papers were sprayed with 5% ethanolic vanillin HCl reagent and then heated in an oven. A pink-red streak developed on all papers extending from the origin to very high R_f values. No single spot was apparent. This paper chromatography ruled out the presence of polyphenolic materials related to benzoic or cinnamic acid. In addition, the paper showed that no polyphenolics of the trihydroxy gallic acid type were present by the absence of a deep-blue color when the ferric chloride reagent was used. The streak indicated polymeric flavanoidal material in the tannin extract.

Leucoanthocyanidin Tests—The tannin extract was soluble in a 1:1 mixture of ethanol–water but was insoluble in water alone. Those natural chromagens that are insoluble in water, or give only colloidal solutions, correspond to condensed polymers (4). A portion of the tannin extract was boiled for 20 min. in a solution of ethanol–HCl. The solution turned from brown to deep red. A spot of an alcoholic solution of the tannin extract was placed on filter paper, and a drop of 5% ethanolic vanillin HCl was added. The paper was heated in an oven, and the spot developed a persistent pink color. Attempts to extract the tannin with ethyl acetate from aqueous solution proved negative. These tests indicated the presence of polymeric flavanoidal 3,4-diols or leucoanthocyanidins in the tannin extract (5, 6).

IR Spectra—An IR spectrum of the tannin extract was made using a KBr pellet. This spectrum was superimposable with one obtained from polymeric leucoanthocyanidins in a tannin extract of wild cherry bark, *Prunus serotina* Erhart. Broad absorption occurred in the 2.9–3.3 μ region. Other major peaks occurred at 6.35 and 6.6 μ.

Anthocyanidins—Condensed tannins consisting of polymeric leucoanthocyanidin units retain the ability to be converted to the individual anthocyanidin units. By this method the polymeric material may be identified. Several grams of the tannin extract was dissolved in ethanol and boiled in a solution of ethanol–HCl for 20 min. The solution changed from brown to deep red. After cooling, the solution was extracted with amyl alcohol. Amyl alcohol selectively extracts anthocyanidins. The amyl alcohol was evaporated *in vacuo* and resulted in a dark-red semicrystalline powder. The red semicrystalline material was dissolved in methanol, and several drops of basic lead acetate were added. A blue precipitate resulted, which was extractable by glacial acetic acid, yielding a deep-red color. A spot of an alcoholic solution of the red material was placed on a filter paper and held over ammonia fumes. A deep-blue color resulted and changed to red when exposed to HCl fumes. These tests were positive for the presence of anthocyanidins (7).

Paper Chromatography of Anthocyanidins—Paper chromatography of the anthocyanidins produced from the polymeric leucoanthocyanidins was carried out on Whatman No. 1 filter paper. The following solvent systems were used: Solvent 1, acetic acid–concentrated HCl–water (10:30:3); Solvent 2, acetic acid–concentrated HCl–water (5:1:5); and Solvent 3, formic acid–3 *N* HCl (1:1). In all three solvent systems, only one red pigment appeared. The R_f values were: Solvent 1, R_f 0.5; Solvent 2, R_f 0.34; and Solvent 3, R_f 0.22. The red pigment in all solvent systems turned deep blue when exposed to ammonia fumes. The R_f values in all three solvent systems were those for 3,5,7,3′,4′-pentahydroxyflavylium chloride (8–10). A commercial sample of the material was obtained,[1] and the paper chromatography was repeated in all three solvent systems.

[1] Obtained from K & K Labs, Plainview, N. Y.
[2] Obtained from S. B. Penick & Co., New York, N. Y.

The R_f values obtained in all solvent systems were identical with that of the commercial sample.

IR and Visible Spectra of Anthocyanidin—An IR spectrum was made of the red pigment and was found to be superimposable with that of a commercial sample of cyanidin chloride. Visible spectrum analysis of the pigment in ethanol–0.1% HCl solution was 545 nm. (11).

DISCUSSION

A tannin extract obtained from powdered cinnamon USP was examined phytochemically. The tannin extract consisted of polymeric leucoanthocyanidin units. Based on the conversion of the polymer to 3,5,7,3′,4′-pentahydroxyflavylium chloride and the identification of the same, it was concluded that the polymer consisted of polymeric units of 5,7,3′,4′-tetrahydroxyflavan-3,4-diol. No monomeric leucoanthocyanidins or other polyphenolic derivatives of benzoic and cinnamic acid were found in the tannin extract.

REFERENCES

(1) L. Buchalter, *J. Pharm. Sci.*, **58,** 10(1969).

(2) L. Buchalter and J. R. Cole, *ibid.*, **56,** 8(1967).

(3) H. W. Youngken, "Textbook of Pharmacognosy," 6th ed., Blakiston, Philadelphia, Pa., 1948, p. 368.

(4) G. M. Robinson and R. Robinson, *J. Chem. Soc.*, **1935,** 744.

(5) E. C. Bate-Smith and N. H. Lerner, *Biochem. J.*, **58,** 122 (1954).

(6) K. Venkataraman, in "Chemistry of Flavanoid Compounds," T. A. Geissman, Ed., Macmillan, New York, N. Y., 1962, p. 76.

(7) L. Hayashi, *Acta Phytochim. Japan*, **117,** 143(1933).

(8) K. Hayashi and Y. Abe, *Bot. Mag. Tokyo*, **69,** 577(1956).

(9) D. G. Roux, *Nature*, **183,** 890(1959).

(10) *Ibid.*, **180,** 973(1957).

(11) J. B. Harborne, *Biochem. J.*, **70,** 22(1958).

ACKNOWLEDGMENTS AND ADDRESSES

Received March 16, 1970, from the *School of Pharmacy, Creighton University, Omaha, NB 68131*

Accepted for publication July 30, 1970.

† Deceased June 7, 1970.

Physiologic Surface-Active Agents and Drug Absorption VIII: Effect of Bile Flow on Sulfadiazine Absorption in the Rat

CHARLES H. NIGHTINGALE*, JAMES E. AXELSON, and MILO GIBALDI

Abstract □ The absorption of sulfadiazine was determined in rat intestinal loops *in situ* under four experimental conditions—*viz.*, control, bile duct ligation, sham bile duct ligation, and sodium dehydrocholate-stimulated bile flow. Enhanced bile flow increased the absorption of the drug about 50%, apparently by increasing the solubility and dissolution rate of sulfadiazine. This possibility is supported by the results of *in vitro* solubility studies. Rats with a ligated bile duct, on the average, showed significantly reduced absorption compared to control levels. The results suggest that bile plays an important, although not critical, role in the absorption of sulfadiazine under these experimental conditions.

Keyphrases □ Sulfadiazine—absorption, bile-flow effect, rats □ Absorption, sulfadiazine—effect of bile flow, rats □ Bile—role in sulfadiazine absorption, rats

Numerous studies have shown that surface-active agents may increase the gastrointestinal absorption of poorly water-soluble drugs by enhancing dissolution *via* an effect on the effective surface area or apparent solubility (1). Since bile manifests considerable surface activity, it is reasonable to consider that bile salts and certain phospholipids, which are normally present in the small intestine, solubilize and enhance the dissolution rate of poorly soluble drugs and should thereby promote the absorption of these compounds. It follows that where there is a diminished bile salt concentration in the proximal intestine, such drugs will be poorly absorbed. This possibility is supported by the findings of Bernhard *et al.* (2) who showed that in the biliary fistula of the rat, less than 1% of a dose of vitamin A was absorbed and that bile administration increased absorption significantly.

Greaves (3) also reported that bile salts are essential for the intestinal absorption of vitamin K in the rat. Adequate absorption of vitamin D by the rat was shown to require bile by Greaves and Schmidt (4). While the bile duct was anastomosed into the colon, the animals absorbed little or none of the vitamin. Oral administration of deoxycholic acid greatly improved absorption of the vitamin in these rats. Taylor *et al.* (5) confirmed the need of bile for adequate vitamin D absorption in dogs. Heymann (6) also found that dogs did not absorb crystalline vitamin D_2 when bile was not present in the small intestine.

Pekanmaki and Salmi (7) studied the gastrointestinal absorption of phenolphthalein and its glucuronide in the cat. Peak blood levels (portal vein) of 50 mcg./ml. were observed in control animals, in contrast to peak levels of 23 mcg./ml. in test animals (ligation of common bile duct) 1 hr. after gastric intubation of phenolphthalein. There was a marked decrease in the absorption of the poorly soluble drug when drainage of bile into the intestine was prevented. However, the absence of bile from the intestine had no effect on the absorptions of the highly water-soluble glucuronide.

More recently, Meli *et al.* (8) reported that endogenous bile influences the rate of intestinal absorption of ethynylestradiol-6,7-^{3}H-3-cyclopentyl ether in rats. The rate of absorption of the estrogen was considerably lower in bile duct-cannulated rats than in control