Insertion of the literature value 0.5 to 0.8 for n gives the form of Eq. 11.

REFERENCES

(1) G. Levy and B. A. Hayes, *New Engl. J. Med.*, **262,** 1053 (1960).

(2) E. Marlowe and R. F. Shangraw, *J. Pharm. Sci.*, **56,** 498 (1967).

(3) W. E. Hamlin, E. Nelson, B. E. Ballard, and J. G. Wagner, *ibid.*, **51,** 432(1962).

(4) D. Cook, H. S. Chang, and C. A. Mainville, *Can. J. Pharm. Sci.*, **1,** 69(1966).

(5) R. O. Searl and M. Pernarowski, *Can. Med. Assoc. J.*, **96,** 1513(1967).

(6) B. Katchen and S. Symchowicz, *J. Pharm. Sci.*, **56,** 1108 (1967).

(7) M. Gibaldi and S. Feldman, *ibid.*, **56,** 1238(1967).

(8) D. E. Wurster and G. P. Polli, *ibid.*, **50,** 403(1961).

(9) G. C. Evans and C. F. Gerald, *Chem. Eng. Progr.*, **49,** 135 (1953).

(10) J. C. Chu, J. Kalil, and W. A. Wetteroth, *ibid.*, **49,** 141 (1953).

(11) C. E. Dryden, D. A. Strang, and A. E. Withrow, *ibid.*, **49,** 191(1953).

(12) P. M. Weinspach, *Chem. Ing. Tech.*, **39,** 231(1967).

(13) M. Pernarowski, W. Woo, and R. O. Searl, *J. Pharm. Sci.*, **57,** 1419(1968).

(14) A. A. Noyes and W. R. Whitney, *J. Am. Chem. Soc.*, **19,** 930(1897).

(15) M. Leva, "Fluidization," McGraw-Hill, New York, N. Y., 1959, p. 247.

(16) P. J. Niebergall and J. E. Goyan, *J. Pharm. Sci.*, **52,** 29 (1963).

(17) A. H. Goldberg, M. Gibaldi, J. L. Kanig, and J. Shanker, *ibid.*, **54,** 1722(1965).

(18) H. Nogami, T. Nagai, and K. Ito, *Chem. Pharm. Bull.*, **14,** 351(1966).

(19) E. L. Parrott and V. K. Sharma, *J. Pharm. Sci.*, **56,** 1341 (1967).

(20) P. Finholt and S. Solvang, *ibid.*, **57,** 1322(1968).

(21) S. J. Rehfeld, *J. Phys. Chem.*, **71,** 738(1967).

(22) M. R. Vidal-Paruta and L. D. King, *J. Pharm. Sci.*, **53,** 1217(1964).

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NOTES

Identification of Monomeric and Polymeric 5,7,3′,4′-tetrahydroxyflavan-3,4-diol from Tannin Extract of Wild Cherry Bark USP, *Prunus serotina* Erhart, Family Rosaceae

LEONARD BUCHALTER

Abstract □ A phytochemical investigation of the tannin content of wild cherry bark USP showed the presence of nonhydrolyzable flavanoidal-type tannins, consisting of monomeric and polymeric leucocyanidin units. Identification was accomplished by paper chromatography, visible and IR spectrophotometry, with commercial samples, and fragmentation by potassium fusion.

Keyphrases □ *Prunus serotina*—tannin extract □ 5,7,3′,4′-Tetrahydroxyflavan-3,4-diol, monomeric, polymeric—extraction, identification □ Paper chromatography—separation, identification □ IR, visible spectrophotometry—identity

Many extracts of drugs from plant sources have a reported tannin content. The word tannin is one that may be applied to several distinct chemical entities. Tannins may consist of the hydrolyzable types which are readily hydrolyzed by acids or enzymes and are therefore classified as gallotannins or ellagotannins. Tannins may also consist of monomeric and polymeric flavan 3,4-diol or 3-ol units. This type of tannin is the nonhydrolyzable or condensed tannin. In many cases the exact chemical nature of the reported tannins has not been evaluated. The purpose of this experiment was to evaluate phytochemically the reported tannin content of wild cherry bark USP.

EXPERIMENTAL

Materials—Paper chromatography was carried out in glass tanks on Whatman No. 1 filter paper. Visible spectra were obtained from a spectrophotometer (Beckman DB). IR spectra were made on a spectrophotometer (Beckman IR 8). Commercial samples of cyanidin (K & K Labs, Plainview, N. Y.) were used. Wild cherry bark USP (S. B. Penick Co., New York, N. Y.) was also used.

Preparation of Extracts—Five hundred grams of coarse, dried wild cherry bark USP, *Prunus serotina* Erhart, Family Rosaceae, was defatted with successive washings of petroleum ether and chloroform. This defatted material was extracted with equal parts of

water and ethanol. The ethanol–water extract was evaporated *in vacuo*, and the brown amorphous residue was taken up in water. The water extract was washed several times with ether, and the ether fraction was discarded. Water was evaporated *in vacuo*, resulting in 10 g. of a light brown scaly-like amorphous residue.

Tannin Tests—To verify the tannin content of the extract obtained, the following tests were carried out: gelatin precipitation test, alkaloidal precipitation test, and 5% alcoholic ferric chloride solution. This extract was positive in all tests for tannins, giving a light blue-gray color with the ferric chloride reagent. A portion of the extract was subjected to hydrolytic procedures in both acidic and alkaline media for several hours. The extract was found to be of the nonhydrolyzable type tannins. Several tests were carried out to determine if the tannin had flavanoidal content.

Leucoanthocyanidins—Five hundred milligrams of the extract was boiled in an aqueous solution containing 10% HCl for 15 min. A deep red color appeared (1). A spot of an aqueous solution of the tannin extract was placed on filter paper and a drop of 5% ethanolic vanillin HCl was added. The spot turned bright pink-red with this reagent. These tests were positive for flavanoidal leucoanthocyanidins, or flavan 3,4-diols (2).

The tannin extract was separated into two fractions. Monomeric leucoanthocyanidins may be extracted from aqueous solution by ethyl acetate, whereas polymers are nonextractable from aqueous solution by this reagent (3). An aqueous extract of the tannin was extracted several times wth ethyl acetate, and the ethyl acetate layer was separated from the aqueous layer. Ethyl acetate extractable material (Fraction 1) and aqueous, nonethyl acetate extractable material (Fraction 2) were then examined for leucoanthocyanidin content by paper chromatography. Five percent alcoholic $FeCl_3$ and 5% ethanolic vanillin HCl were used as chromogenic sprays. Solvent systems used were: Solvent 1 (10% acetic acid); Solvent 2 (water); Solvent 3 (*n*-butanol–acetic acid–water 4:1:5 organic phase); Solvent 4 (*n*-butanol–acetic acid–water 4:1:5 aqueous phase); Solvent 5 (amyl alcohol–acetic acid–water 4:1:5); Solvent 6 (*n*-butanol–acetic acid–water 8:2:2). Fraction 1 showed the presence of a monomeric leucoanthocyanidin in all solvent systems. It was colorless visibly and nonfluorescent when examined in UV light. Monomers were detected on spraying the developed papers with the ethanolic vanillin HCl reagent, and gave a bright pink-red spot at the R_f of the monomer, which persisted for several days. When sprayed with $FeCl_3$ reagent, the spots gave a blue-gray color.

R_f values were: Solvent 1, R_f 0.57; Solvent 2, R_f 0.59; Solvent 3, R_f 0.74; Solvent 4, R_f 0.75; Solvent 5, R_f 0.53; Solvent 6, R_f 0.78. Only one spot was present in all systems, which indicated the presence of a single monomer in Fraction 1. Fraction 2 was examined in the same solvent systems, and in all cases, when sprayed with ethanolic vanillin HCl reagent, showed only a bright pink-red streaking from the origin, indicative of polymeric leucoanthocyanidins. Spraying with $FeCl_3$ reagent indicated the absence of gallic acid type phenolics, since no dark blue color developed. This reagent also indicated that the polymeric material consisted of a single type of phenolic, since the spray gave a consistent light gray reaction throughout.

Anthocyanidins—Proof of structure of leucoanthocyanidins has been performed by converting them to their corresponding flavylium salts by boiling in mineral acid solution. A portion of Fraction 1, a straw-like yellow-colored amorphous material, was boiled for 15 to 20 min. in 10% ethanol HCl. The solution which turned deep red was allowed to cool and was then extracted with amyl alcohol. Upon evaporation of the amyl alcohol, a dark pink-red crystalline material resulted. This material was exposed to several qualitative tests to determine its anthocyanidin content. An aqueous solution gave a blue precipitate with basic lead acetate solution. A spot of an alcoholic solution of the red material on filter paper turned deep blue when exposed to ammonia fumes, and returned to pink-red when exposed to concentrated HCl fumes. These tests were positive for anthocyanidins (4).

Preparative paper chromatography of the red material was carried out on twenty sheets of Whatman No. 1 filter paper. A solvent system, consisting of water–acetic acid–concentrated HCl (10:30:3), was used to develop the papers. Papers were streaked at the origin with an alcoholic solution of the pink-red extract. A pink-red pigment separated out at R_f 0.50. Only one red pigment was apparent. When the red streak was exposed to ammonia fumes, it turned dark blue. This material was carefully cut from the twenty papers and extracted for several days with a methanolic 10% HCl solution and filtered. The solvent was evaporated and a crystalline pink-red material resulted. The pink-red crystalline material was exposed to paper chromatography in three solvent systems: Solvent 1 (acetic acid–concentrated HCl–water 5:1:5); Solvent 2 (water–acetic acid–concentrated HCl 10:30:3); Solvent 3 (formic acid–3 *N* HCl 1:1). A red pigment appeared in all solvent systems at the following R_f values: Solvent 1, R_f 0.34; Solvent 2, R_f 0.50; Solvent 3, R_f 0.22. These R_f values are identical with those shown in the literature for cyanidin chloride (5–7).

Paper chromatography was rerun in all three solvent systems with a commercial sample of cyanidin chloride (K & K Labs, Plainview, N.Y.), and R_f values of the commercial sample and pigment were identical in all systems. An IR spectrum was made of the pigment, and was found to be superimposable with that of a commercial sample of cyanidin chloride. IR showed broad absorption at 2.9 to 3.2 μ, peaks at 6.27, 6.7, and 7.3 μ. Visible spectra absorption in ethanol 0.1% HCl was 545 mμ and a bathochromic shift to 563 mμ upon addition of several drops of ethanolic aluminum chloride (8).

Potassium Fusion—The isolated pigment was then fused by a method of potassium fusion (9) and the phenolic portion yielded phloroglucinol, while the acidic portion yielded protocatechuic acid. These fragments were identified by TLC on silica gel layers (10).

DISCUSSION

The presence of tannins has been reported in wild cherry bark USP, but the exact chemical nature of the tannins had not been determined prior to this experiment. The tannin extract was shown to consist of monomeric and polymeric flavanoidal units consisting of monomeric and polymeric leucocyanidin.

Based on the identification of (3,5,7,3′,4′-pentahydroxyflavylium chloride) from Fraction 1, it was concluded that the monomeric leucoanthocyanidin (5,7,3′,4′-tetrahydroxyflavan-3:4-diol) was present in the original tannin extract.

The water-soluble nonethyl acetate extractable material (Fraction 2) also yielded only cyanidin chloride upon boiling in concentrated mineral acid. This showed that the polymeric material consisted of polymeric leucocyanidin. The polymers often accompany the monomers in tannin extracts.

REFERENCES

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

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

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

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

(5) K. Hayashi and Y. Abe, *Botan. Mag. Tokyo*, **69,** 577(1956).

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

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

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

(9) T. A. Geissman, "The Chemistry of Flavanoid Compounds," Macmillan, New York, N. Y., 1962, pp. 259–260.

(10) K. Randerath, "Thin Layer Chromatography," Academic, New York, N. Y., 1963, p. 178.

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