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THE *p*-HYDROXYCINNAMYL COMPOUNDS OF SIAM BENZOIN GUM

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Abstract—Siam Benzoin gum and coniferyl benzoate isolated from the gum were reduced with lithium aluminium hydride. In addition to the anticipated coniferyl alcohol, *p*-coumaryl alcohol was isolated from the phenolic fraction and it was found that the benzoate in Siam Benzoin gum contains 15 per cent *p*-coumaryl alcohol; sinapyl and caffeyl alcohol were shown to be absent. By use of a lithium aluminum hydride reduction coniferyl alcohol can be obtained directly from Siam Benzoin gum, whereas attempted saponification only results in polymerization of the benzoate ester.

INTRODUCTION

SIAM BENZOIN gum is a water-insoluble resin obtained from the tree, *Styrax benzoin* Dryand (Styracaceae). This gum was first thoroughly investigated by Reinitzer,[1] who found the major constituent to be coniferyl benzoate, comprising 75–80 per cent of the gum. A small amount (2·3 per cent) of cinnamyl benzoate and 12 per cent free benzoic acid were also found. The presence of other hydroxycinnamyl compounds was excluded from consideration since the individual constituents analyzed totaled 100 per cent.

Other than as the benzoate ester in Siam Benzoin gum, coniferyl alcohol only occurs in nature as coniferin, a glucoside present in the cambium of all ligneous plants and the source of the coniferyl units in lignin.[2] Free coniferyl alcohol can be obtained from coniferin through cleavage of the glycosidic linkage with an enzyme preparation,[3] but no such simple isolation from coniferyl benzoate has been achieved. All attempts to hydrolyze the ester have resulted in polymerization regardless of how mild the conditions of saponification.[4] This result is unusual in that, although the free coniferyl alcohol readily polymerizes under acidic conditions, it is relatively stable in alkaline medium. Freudenberg and Bittner[4] did isolate coniferyl alcohol from the benzoate ester after tosylation of the free phenolic hydroxyl followed by saponification of the benzoate and tosylate esters, in that order. The lignin sulfonate monomers, vinylvanillyl sulfonate and coniferyl sulfonate, were synthesized by a bisulfite treatment of coniferyl benzoate from Siam Benzoin gum.[5–7] However, when an alkaline sulfonation was attempted no monomer sulfonates appeared to be present but an insoluble residue was formed instead.[8]

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[1] F. REINITZER, *Arch. Pharm.* **259**, 1 (1921); **259**, 60 (1921); **263**, 347 (1925); **264**, 131 (1926).

[2] W. KARRER, *Konstitution and Vorkommen der organischen Pflanzenstoffe*, p. 108. Birkhäuser Verlag, Basel (1958).

[3] F. TIEMANN, *Chem. Ber.* **7**, 608 (1874); **8**, 1127 (1875); **11**, 659 (1878).

[4] K. FREUDENBERG and F. BITTNER, *Chem. Ber.* **83**, 600 (1950).

[5] G. M. ANDRUS, Ph.D. thesis, University of Washington, Seattle, Washington (1957).

[6] S. W. SCHUBERT, Ph.D. thesis, University of Washington, Seattle, Washington (1961).

[7] D. W. GLENNIE and J. S. MOTHERSHEAD, *Tappi* **47**, 356 (1964).

[8] D. W. GLENNIE, *Tappi* **49**, 237 (1966).

Since Siam Benzoin gum is obtained, through injury to the cambium, from a tree that belongs to a high order (the Ebenales) of angiosperms other hydroxycinnamyl compounds besides coniferyl benzoate might be expected to be present. The lignin of angiosperms is composed of about equal amounts of coniferyl and sinapyl units, and consequently sinapyl benzoate could be present in the gum. *p*-Coumaryl and caffeyl compounds are widespread among plants[9] and might also be expected in the gum. In addition, *p*-coumaryl alcohol, to the extent of 14 per cent, is a building unit of gymnosperm lignin, a lignin which is predominantly composed of coniferyl units;[10, 11] and caffeyl alcohol is considered the immediate precursor of coniferyl alcohol.[12]

Siam Benzoin gum could be used as an inexpensive source of coniferyl alcohol if its benzoate is the only hydroxycinnamyl constituent. Lithium aluminium hydride reduction of coniferyl benzoate instead of saponification should yield coniferyl alcohol and benzyl alcohol. Using the gum itself as starting material would avoid the tedious task of isolating the coniferyl benzoate, total yields of which are as low as 13–20 per cent or only one-quarter of the coniferyl benzoate available in the gum.[4] The separation of coniferyl alcohol from the other substances present after reduction of the Siam Benzoin gum would be simplified, since coniferyl alcohol would be the only phenolic substance in the reaction mixture. Although not exactly comparable, the lithium aluminium hydride reduction of ethyl ferulate gives coniferyl alcohol in 90 per cent yield; and therefore, the best anticipated yield with Siam Benzoin gum as starting material would be 45 per cent.

RESULTS AND DISCUSSION

Lithium aluminium hydride reduction of Siam Benzoin gum gave a neutral and a phenolic fraction. Aqueous sodium bicarbonate treatment of the latter removed only minute amounts of material, indicating an absence of carboxylic acids. The neutral fraction gave an u.v. spectrum nearly identical with that of benzyl alcohol and appears to be fairly pure benzyl alcohol. The u.v. spectrum of the phenolic fraction was the same as that of coniferyl alcohol; however, paper chromatography indicated that two other substances were also present.

The two isolated fractions, together with known phenolic alcohols, were chromatographed on paper using xylene-dimethylformamide.[13] The R_f values of the reference standards and the colors they develop with diazotized 2-amino-4-nitroanisole are given in Table 1. The phenolic fraction had three constituents that gave positive reactions with the spray reagent. Two of these had the same R_f and color reactions as coniferyl and *p*-coumaryl alcohols respectively; a third substance of R_f 0·19, present in only small amount, might possibly be a reduction product of Reinitzer's *d*-siaresinolic acid.[1] The Mäule test[14] for syringyl and sinapyl alcohols was negative in both neutral and phenolic fractions, as was also the test for protocatechuic and caffeyl alcohols.[15] By performing limits-of-sensitivity tests, it was determined that sinapyl and caffeyl alcohols could be detected if they were present to the

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[9] E. C. BATE-SMITH in *Wood Extractives* (edited by W. E. HILLIS), p. 134. Academic Press, New York (1962).

[10] K. FREUDENBERG, in *The Formation of Wood in Forest Trees* (edited by M. H. ZIMMERMANN), p. 207. Academic Press, New York (1964).

[11] K. FREUDENBERG, *Science* **148**, 595 (1965).

[12] A. C. NEISH, in *The Formation of Wood in Forest Trees* (edited by W. H. ZIMMERMANN), p. 220. Academic Press, New York (1964).

[13] K. FREUDENBERG and B. LEHMANN, *Chem. Ber.* **93**, 1354 (1960).

[14] F. E. BRAUNS and D. A. BRAUNS, *The Chemistry of Lignin*, Supplement Volume, p. 52. Academic Press, New York (1960).

[15] H. A. SCHROEDER, *J. Chromatog.*, In Press.

extent of 2–3 per cent in the phenolic fraction. Although the odor of vanillin can be detected in the original Siam Benzoin gum and Reinitzer[1] states its presence in the gum up to 0·3 per cent, its reduction product, vanillyl alcohol, could not be detected. The ratio of *p*-coumaryl alcohol to coniferyl alcohol in the phenolic fraction was such that 15 per cent (±2%) of the total amount was *p*-coumaryl alcohol. Comparisons in triplicate of the phenolic fraction obtained from several Siam Benzoin gum reductions showed this 15 per cent value to be accurate within 2–3 percentage points. By means of preparative paper chromatography the two substances were isolated, and purified. A comparison of their u.v. spectra in neutral and alkaline aqueous solutions with authentic samples verified their identification as *p*-coumaryl and coniferyl alcohols. The presence of *p*-coumaryl alcohol precluded the development of other, simpler procedures, for the isolation of pure coniferyl alcohol from the reduction of Siam Benzoin gum. The total yield of phenolic alcohol was 32·4 per cent, representing 75 per cent recovery of the benzoates in the original gum.

TABLE 1. PAPER CHROMATOGRAPHY OF PHENOLIC ALCOHOLS IN XYLENE–DIMETHYLFORMAMIDE (9:2)

Compound	R_f value	Color reaction*
Sinapyl alcohol	0·34	deep blue†
Coniferyl alcohol	0·42	purple
p-Coumaryl alcohol	0·46	deep red
Caffeyl alcohol	0·22	indigo†
Syringyl alcohol	0·30	pink†
Vanillyl alcohol	0·36	orange
p-Hydroxybenzyl alcohol	0·40	yellow
Protocatechuic alcohol	0·21	red violet†

* After spraying with 0·05 per cent diazotized 2-amino-4-nitroanisole in 80 per cent aqueous methanol and 0·1 N NaOH.

† Rapidly fades, resulting in a gray-tan to white spot.

The reduction of coniferyl benzoate isolated from Siam Benzoin gum gave the same results except that the amount of *p*-coumaryl alcohol in the phenolic fraction was less than that obtained by the direct reduction of Siam Benzoin gum. Of the total amount of *p*-hydroxycinnamyl alcohols present, only 9 per cent was *p*-coumaryl alcohol. Values given in the literature for the methoxyl content of coniferyl benzoate isolated from Siam Benzoin gum are 0·66–0·99 percentage points lower than the value calculated for this compound.[1, 16] If this discrepancy were due to the presence of *p*-coumaryl benzoate in the material, the amount of *p*-coumaryl benzoate necessary to lower the methoxyl content to this extent would be 6·1–9·1 per cent. The alkaline polymerization product of coniferyl benzoate also has a methoxyl content lower than expected.[4]

Paper chromatography of an ethereal solution of Siam Benzoin gum or of coniferyl benzoate isolated from it showed the absence of any of the phenolic alcohols shown in Table 1. The only phenolic compound present was coniferyl benzoate, with an R_f of 0·75. Numerous attempts to separate the mixture of *p*-coumaryl and coniferyl benzoates presumed to be present in the gum by means of paper chromatography were unsuccessful.

Obtaining coniferyl alcohol by a lithium aluminium hydride reduction of coniferyl benzoate is not exactly comparable to a similar reduction of ethyl ferulate; however, the high yields of coniferyl alcohol obtained here indicate a similarity in reactivity under the conditions used.

[16] A. ZINKE and J. DZRINAL, *Monatsh. Chem.* **41**, 431 (1920).

EXPERIMENTAL

Reference compounds. p-Coumaryl, caffeyl, coniferyl, and sinapyl alcohols were prepared by the procedures of Freudenberg.[17–21] The phenolic hydroxyl was not acetylated or otherwise substituted before reduction. In the preparation of caffeyl alcohol no special precautions were taken other than repression of *o*-quinone formation in aqueous alkaline solution by prior addition of sodium borohydride.[22] All products readily crystallized except sinapyl alcohol, which remained a viscous, unstable, oily solid. Yields and melting points were as follows: *p*-coumaryl alcohol, 91%, m.p. 124°;[17] caffeyl alcohol, 90%, m.p. 132°;[21,23] coniferyl alcohol, 92%, m.p. 73°;[20] and sinapyl alcohol, 45%, lit. m.p. 66–67°.[19,20] Vanillyl, syringyl, protocatechuic, and *p*-hydroxybenzyl alcohols were prepared by $NaBH_4$ reduction of their respective aldehydes.

Reduction of Siam Benzoin gum. The Siam Benzoin gum tears* were dried for one week in a vacuum desiccator over KOH pellets. The dry gum (10·00 g) was dissolved in 400 ml absolute ether, and the insoluble foreign matter was removed by filtratration under dry N_2. In a separate operation in dry N_2, powdered $LiAlH_4$(5·00 g) was added to 400 ml absolute ether, previously cooled to −15°. To this suspension of $LiAlH_4$ in ether was added dropwise (over 3 hr) under N_2 at −5° with stirring the ethereal gum solution. The reaction mixture was stirred overnight under N_2 and the temperature allowed to rise to ambient.

The reaction mixture was again cooled to −15° and an aqueous $NaBH_4$ solution (6 g/40 ml H_2O) was *cautiously* added dropwise with vigorous stirring.[22] The temperature was kept below −5° and a N_2 atmosphere maintained and 400 ml of water was also added. Immediately after decomposition of the excess $LiAlH_4$ was complete, the entire mixture was transferred to a 2 l. separatory funnel. The ether layer was separated from the aqueous fraction and the aqueous fraction extracted twice with 150 ml portions of ether. The combined ether fractions were washed twice with 150 ml portions of water and the wash water added to the aqueous fraction. The pH of the aqueous solution was carefully adjusted to 6 by the dropwise addition of 1N HCl while the solution was being gently stirred or shaken under a layer of 400 ml ether. (Care was exercised to prevent excessive foaming due to decomposition of the $NaBH_4$.) After neutralization, the ether layer was immediately removed and the aqueous solution extracted twice with 150 ml portions of ether. The combined ethereal solutions were extracted twice with 100 ml portions of aqueous 0·1 N $NaHCO_3$ and dried with anhydrous $MgSO_4$. Material recovered after evaporation of the ether under a gentle stream of N_2 gas totaled 3·24 g and the yield, based on original Siam Benzoin gum, was 32.4 per cent. Neutral and alkaline aqueous u.v. spectra were obtained of the residue from both the neutral ether fraction and the phenolic ether fraction with a Beckman DB Spectrophotometer.

Isolation and reduction of coniferyl benzoate from Siam Benzoin gum. The procedure of Freudenberg and Bittner was followed for the isolation of coniferyl benzoate.[4] Yield, based on original gum tears, was 27 per cent and after further purification through several recrystallizations, 17 per cent. The reduction of this coniferyl benzoate and isolation of the products follow the above described procedure for Siam Benzoin gum. Yield of phenolic substances was 5·53 g from 10 g of coniferyl benzoate or 87·3 per cent calculated as coniferyl alcohol.

Identification of products. The reference compounds and the phenolic substances obtained by reduction were chromatographed by the descending method using Whatman No. 1 filter paper. The paper was impregnated with dimethylformamide before application of the sample and developed with xylene–dimethylformamide (9:2).[13] The substances were detected by (1) u.v. fluorescence in presence of ammonia and by (2) formation of colored reaction products with a diazo dye. Paper chromatograms were sprayed with 0·05 per cent diazotized 2-amino-4-nitroanisole† in 80 per cent aqueous methanol, air-dried, and then sprayed with 0·1 N NaOH. Syringyl and sinapyl alcohols were detected by modifying the Mäule test[14] for use with paper chromatography. The chromatogram was fumed with Cl_2 gas in a closed container for 5 minutes followed by spraying with a 10 per cent aqueous Na_2SO_3, which produced a bright pink to red color. Protocatechuic and caffeyl alcohols were detected by use of phoroglucinol and alkali.[15]

Preparative paper chromatography was utilized to isolate the individual phenols. After removal of the strips containing the individual compounds from the remainder of the paper chromatogram, they were further dried under water aspirator vacuum at 45° for several days. The individual compounds were eluted from the paper by soaking in a minimum amount of ethanol. The ethanolic solution was added to 40 ml of H_2O and extracted with three 10 ml portions of ether. The combined ether extracts were, in turn, extracted with two

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* Benzoin U.S.P. Siam from Fritzsche Brothers, Inc., 76 9th Ave., New York, N.Y. 10011.

† Fast Scarlet R from Koppers Co., Inc., Koppers Building, Pittsburgh, Pa. 15219.

[17] K. FREUDENBERG and G. GEHRKE, *Chem. Ber.* **84**, 443 (1951).

[18] K. FREUDENBERG and R. DILLENBURG, *Chem. Ber.* **84**, 67 (1951).

[19] K. FREUDENBERG, R. KRAFT and W. HEIMBERGER, *Chem. Ber.* **84**, 472 (1951).

[20] K. FREUDENBERG and H. H. HÜBNER, *Chem. Ber.* **85**, 1181 (1952).

[21] K. FREUDENBERG and W. HELL, *Chem. Ber.* **86**, 190 (1953).

[22] H. A. SCHROEDER, *Phytochem.* in press.

[23] K. HERMANN, *Pharmazie* **8**, 303 (1953).

10 ml portions of 1N NaOH. This alkaline solution was neutralized with HCl and extracted with two 10 ml portions of ether. This ethereal solution was evaporated to dryness, and the u.v. spectra of the residues determined in neutral and alkaline aqueous media and compared with the appropriate reference compounds.

Paper chromatographic determination of the ratio of p-*coumaryl alcohol to coniferyl alcohol.* Standard solutions containing 10 mg/ml coniferyl alcohol enriched with 6, 8, 10, 12, 14, 16, 18 and 20 per cent of *p*-coumaryl alcohol were prepared. The concentration of the isolated phenolic fractions from the reduction reactions was adjusted so that the amount of coniferyl alcohol present would match that of coniferyl alcohol in the standard solutions. The standard solutions were alternated, in increasing concentration of *p*-coumaryl alcohol, with the phenolic fractions on paper chromatograms. After development and spraying of the chromatograms the intensity of the *p*-coumaryl alcohol spot was visually compared between the samples present and that standard which best approximated the isolated phenolic fraction.

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