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SHORT COMMUNICATION

ISOLATION AND CHARACTERIZATION OF a-GUAIACONIC ACID AND THE NATURE OF GUAIACUM BLUE*

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Abstract-a-Guaiaconic acid, the constituent of gum guaiac resin that turns blue upon oxidation, was purified by elution from a polyamide column with aqueous formic acid and shown to be 2,5-di-(4-hydroxy-3-methoxyphenyl)-3,4-dimethylfuran. The blue product, guaiacum blue, is the bis-methylenequinone obtained by twofold one-electron oxidation of a-guaiaconic acid.

GUAIACUM or lignumvitae are names given to timber from a tropical-American genus of trees (*Guaiacum sanctum* L. or G. *officinale* L.) (Zygophyllaceae). The wood is well known for its great hardness and density (spec. gr. = 1·17–1·33) due to its interlocking diagonal and oblique fibers and high content (15-30 %) of resin in the green-brown heartwood. The resin is removed from the logs as a melt or from the chipped wood by boiling in seawater; it is marketed as brownish lumps, m.p. cu. 85–90°, called gum guaiac.

The resin or the wood itself formerly had many medical and pharmacological applications, e.g. in treating chronic ailments such as gout or rheumatism, in tests for occult blood in stains or gastric contents (ether solution turns blue), and in Schonbein-Pagenstecher's test paper for detecting traces of HCN (guaiac + $CuSO_4$ + HCN give blue color). However, neither the resin nor the wood is now included in the USP.

Tincture of guaiac turns blue in the presence of many inorganic or organic oxidizing agents and has long been used to detect oxidative enzymes such as laccase (*p*-diphenol:O_2 oxidoreductase, E.C. 1.10.3.2), tyrosinase (*o*-diphenol : O_2 oxidoreductase, E.C. 1.10.3. 1), or peroxidase (donor: H_2O_2 oxidoreductase, E.C. 1.11.1.7). The active principle is a hitherto unknown compound, classically named a-guaiaconic acid. King and Wilson' characterized nine phenolic lignans in gum guaiac by alkylating the whole resin and studying methylated and ethylated derivatives then isolated; their technique indicated the whereabouts of the initial phenolic functions, but could not help to establish which compound (if any) was a-guaiaconic acid. We have now obtained the active constituent.9

Preliminary experiments with TLC and paper chromatography of resin extracts established that a-guaiaconic acid was a single rather minor constituent of the resin that

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§ Details of all work with a-guaiaconic acid and guaiacum blue can be found in the Ph.D. thesis of JOHN F. KRATOCHVIL, University of Wisconsin (1969).

[1] F. E. KING and J. G. WILSON, *J. Chem. Soc.* 4011 (1964).

fluoresced blue under UV light and could be visualized on chromatograms by spraying with tyrosinase or KIO_4. It autoxidized or photolysed readily on thin layer plates to give guaiacum blue.

Purified a-guaiaconic acid is readily autoxidizable, especially in alkaline solution, and gives the characteristic deep blue when aqueous tyrosinase or KIO_4 is added to its alcoholic solution. The IR spectrum of a-guaiaconic acid (KBr disk) showed the bands in cm^{-1}: 3440(s) OH, 3002(w), and 2940(w) CH, 1612(w), 1596(w), and 15 15(s) Ar, 1468(m), and 1455(m) CH_3, 1430(m), 1402(w), 1363(w), 1325(w), 1280(m), 1263(s), $ArOCH_3$, 1213(s), and 1200(s) ArOH, 1177(m), 1119(m), 1041(m), 1030(w), 895(w), 850(w), 841(w), 818(w), 790(m), 718(w), 682(w). The absence of carbonyl absorption and our observation that a-guaiaconic acid was soluble in dilute alkali, but precipitated on lowering the pH to 10 indicated that this compound was a phenol rather than an acid.

A UV spectrum of a-guaiaconic acid in 95 % EtOH showed intense absorption at 324 nm (log ϵ 4·44), with a minor peak at 251 nm. Addition of sodium ethoxide shifted the longwave maximum to 342 nm and the minor peak to 275 nm; acidification returned the maximum to 324 nm. No shift was observed on addition of sodium acetate. These observations indicate a highly conjugated phenolic molecule.

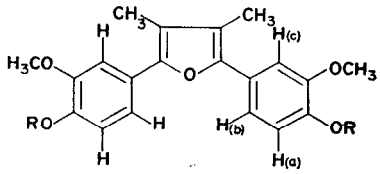
The NMR spectrum of a-guaiaconic acid in perdeuteroacetone (100 MHz) showed the following resonances: methyl (δ = 2·18 vs. TMS, s, integration ratio = 3), phenolic OH (2·80, s, 1), methoxyl (3.90, s, 3), aromatic (6·89, d, J = 8·0 Hz, H_a; 7·15, q, J = 8·2 Hz, H_b; 7·25, d, J = 2·0 Hz, H_c; together 3). When D_2O was added, the phenol peak disappeared.

A mass spectrum of a-guaiaconic acid exhibited a stable molecular ion at m/e = 340 and prominent ion peaks at m/e = 325, 297 and 189. Metastable peaks at m/e = 310·66 and 271.41 indicated that the molecular ion lost successively a methyl radical and carbon monoxide to give the ions at m/e = 325 and 297. This fragmentation is characteristic of guaiacyl compounds.[2] The ion at m/e = 189 may be produced by loss of 151 mass units from the molecular ion. A mass unit of 151 corresponds to a hydroxymethoxybenzoyl radical, indicative of an a-substituted furan compound.[3,4]

A compound[1] isolated from methylated gum guaiac called dimethylfuroguaiacin (I) had a UV maximum at 326 nm (log ϵ = 4·47) and fluoresced bright blue; the location of the original phenolic group was established by examining diethylfuroguaiacin (II) isolated from ethylated resin. This plus the facts that furoguaiacin (III) would have a molecular weight of 340 and that all of our data are compatible with a compound of structure III suggest that a-guaiaconic acid is 'furoguaiacin'.

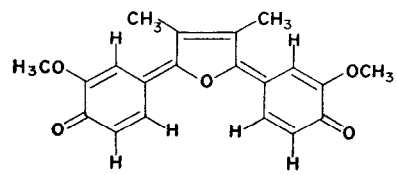
Confirmation of the structure of a-guaiaconic acid was obtained by methylation with CH_2N_2, followed by crystallization from ethanol-acetone-water. The white crystals of the dimethyl ether produced displayed bluish fluorescence under UV light, had a strong absorbance at 324 nm (95 % ethanol; no base shift), and had a m.p. of 170–171°. The m.p.[1] of dimethylfuroguaiacin was given as 169-170". NMR spectrum of our dimethyl derivative (in $CDCl_3$) showed loss of the phenolic absorption of a-guaiaconic acid and the presence of two methoxyl peaks of equal intensity at 3·90 and 3.92 δ. A mass spectrum of our I showed a molecular ion at m/e of 368 and two significant fragment peaks at m/e = 353 and 184.

[2] C. S. BARNES and J. L. OCCOLOWITZ, *Australian J. Chem.* **16, 219 (1963).**
[3] J. COLLIN, *Bull. Soc. Chim. Belges 69, 449 (1960).*
[4] K. HEYNS, R. STUTE and H. SCHARMANN, *Tetrahedron 22, 2223 (1966).*



(I-III)

I R = CH_3, dimethylfuroguaiacin;
II R = C_2H_5, diethylfuroguaiacin;
III R = H, a-guaiaconic acid;



(IV)

IV Guaiacum blue.

The pathway leading to the latter is not known, but the ion of mass 353 results from the loss of a methyl radical and the formation of a stable allylic ion.

Oxidation of the phenol with a variety of enzymes or inorganic oxidants produced the characteristic blue, water-insoluble pigment guaiacum blue (ϵ_{max} in benzene 580 nm, in chloroform 585 nm). Failure to obtain an ESR signal from the pigment, its rapid decoloration by acids or base, and the regeneration of color on treatment with weak base of a solution decolored with HCI strongly suggest that guaiacum blue is the highly conjugated *bis*-methylenequinone (IV).

EXPERIMENTAL

Pulverized gum guaiac (25 g) was triturated int he dark with 400 ml of EtOH acidified with 2 ml of 50% HOAc, filtered, and re-extracted with a further 100 ml of acidified solvent. The combined extract was centrifuged, evaporated in *vacuo*, and dried by evaporation with two 100-ml portions of benzene. Repeated dissolution of the residue in $CHCl_3$ and precipitation with benzene, and subsequent dissolution of the contents of the supernatant in benzene and precipitation with hexane removed polar constituents of the exact, and left 11.3 g of red-yellow viscous oil enriched in a-guaiaconic acid. The oil (10 g) was chromatographed in the dark on 120 g of purified polyamide[5] using 40% aq. HCOOH as eluent and yielded about 024 g of crude a-guaiaconic acid, which after three recrystallizations from acetone–HOAc–H_2O gave white needles, m.p. 149" (yield from resin 1.4%).

Analysis (C, 70·63; H, 5.99; OMe, 18.19; calculated for $C_{20}H_{20}O_5$.C, 7057; H, 5.92; OMe, 18·24), the molecular weight (340 by mass sprectoscopy), and the NMR data show that a-guaiaconic acid is a symmetrical guaiamonoepoxylignan.

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[5] M. K. SEIKEL, F. D. HOSTETTLER and D. B. JOHNSON, *Tetrahedron 24, 1475* (1968).