Chemical Study on *Haematoxyylon campechianum*: a Sweet Principle and New Dibenz[b,d]oxocin Derivatives

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The sweet principle of an extract of heartwood of *Haematoxyylon campechianum* was identified as hematoxylin (1), the well-known staining reagent already isolated from this plant. In addition to 1, two new dibenz[b,d]oxocin derivatives (2 and 3) were obtained. The structure of 2, named hematoxylin A, was elucidated as 3,4,10,11-tetrahydroxy-7,8-dihydro-6H-dibenz[b,d]oxocin-7-one. The latter compound (3) was purified as a tetramethyl ether (3') named tetra-O-methylhematoxylin B and its structure was assigned as 7-hydroxy-3,4,10,11-tetramethoxy-7,8-dihydro-6H-dibenz[b,d]oxocin-7-methanol.

**Keywords** *Haematoxyylon campechianum*; Leguminosae; logwood; sweet principle; hematoxylin; hematoxylin A; tetra-O-methylhematoxylin B; dibenz[b,d]oxocin

*Haematoxyylon campechianum* L. (Leguminosae) is a well-known tree named “logwood,” growing in Mexico, the West Indies and South America. Heartwood of this tree is a source of mordant dye. It is also known that extract of the heartwood tastes sweet, and it has been used as a sweetener. The present paper reports the identification of a sweet principle of this tree. Isolation and structure elucidation of two new dibenz[b,d]oxocin derivatives are also described.

The dried and powdered heartwood was extracted with 50% methanol and the sweet extract was chromatographed on highly porous synthetic polymer with water, 30% ethanol, 50% ethanol, ethanol and finally acetone. Of these eluates, the 30% ethanolic eluate tasted sweet. This eluate was chromatographed on silica gel, affording three fractions, tentatively designated as frs. 1—3. Rechromatography of the sweet fraction, fr. 2, on silica gel yielded a sweet crystalline compound (1). The bitter fraction, fr. 1, afforded a crystalline compound (2) on silica gel chromatography followed by high performance liquid chromatography (HPLC). The tasteless fraction, fr. 3, was subjected to chromatography on silica gel followed by HPLC to give a substance (3) which was further purified as its methyl ether, affording the pure compound (3').

By inspection of the nuclear magnetic resonance (NMR) spectrum and fast-atom bombardment mass spectrum (FAB-MS), the sweet principle (1) was identified as hematoxylin, which has already been isolated from this plant. Hematoxylin is a well-known staining reagent in biology, but this is the first report of the sweetness of this compound. Hematoxylin is rapidly oxidized to give a red pigment named hematein (4), which was found to be tasteless. Brazilin (5), a congener of 1, has been isolated from *Caesalpinia echinata* (Leguminosae), but it is tasteless. This indicates an important role of the 4-hydroxyl group of this skeleton for sweetness.

The elemental analysis of compound 2 coupled with the positive FAB-MS ([M+H]+: m/z 289) led to the molecular formula C_{15}H_{12}O_{6}. The infrared (IR) spectrum (KBr) of 2 showed a carbonyl absorption band at 1700 cm^{-1}. The 1H-NMR spectrum of 2 showed two methylene signals at δ 3.30 and 4.40, each as a 2H singlet. The 13C-NMR spectrum of 2 (Table I) exhibited two methylene and one carbonyl carbon signals. The 13C-1H correlation spectroscopy (13C-1H COSY) spectrum established the correlation of these methylene carbon and proton signals. The 13C-1H long range COSY spectrum indicated the presence of long range coupling between the carbonyl carbon signal and the two methylene proton signals. Based on these results, a partial structure A (Chart 2) was proposed for 2.

The 1H-NMR spectrum of 2 showed a pair of ortho-located aromatic proton signals at δ 6.53 (1H, d, J = 8.3 Hz), 6.69 (1H, d, J = 8.3 Hz) and aromatic proton signals having

![Chart 1](image1)

![Chart 2](image2)

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no vicinal proton at $\delta$ 6.62 (1H, s) and 6.65 (1H, s). The $^{13}$C-NMR spectrum of 2 (Table I) exhibited twelve aromatic carbon signals and the distortionless enhancement by polarization transfer (DEPT) experiment disclosed that the aromatic carbon signals at $\delta$ 111.8, 116.4, 116.5 and 117.8 (in dimethyl sulfoxide-d$_6$ (DMSO-d$_6$)) are due to carbons bearing a proton and others are due to carbons without any proton. The $^1$H-1H COSY and $^{13}$C-1H COSY spectra furnished the correlations of these proton and carbon signals. The $^{13}$C-1H long range COSY spectrum revealed the presence of long range coupling between the following carbon and proton signals: a carbon signal at $\delta$ 126.4 with proton signals at $\delta$ 6.69 and 6.62; a carbon signal at $\delta$ 130.0 with proton signals at $\delta$ 6.53 and 6.65. In the nuclear Overhauser effect (NOE) differential spectrum, NOE was observed between the proton signals at $\delta$ 6.53 and 6.62. These observations led to a biphenyl-type partial structure B as illustrated in Chart 2.

The connection of the two partial structures A and B was established as follows. In the $^{13}$C-1H long range COSY spectrum, long range coupling was observed between a methylene proton signal at $\delta$ 3.30 and an aromatic carbon signal at $\delta$ 122.8 and also between a methylene proton signal at $\delta$ 4.40 and an aromatic carbon signal at $\delta$ 144.2. The NOE differential spectrum demonstrated the presence of NOE between a methylene proton signal at $\delta$ 3.30 and an aromatic proton signal at $\delta$ 6.65. Compound 2 was thus formulated as 3,4,10,11-tetrahydroxy-7,8-dihydro-6H-dibenzo[b,d]oxocin-7-one (Chart 2). Isolation of a dibenz[b,d]oxocin derivative was first reported by Nagai et al., who isolated a compound named protosapannin A (6) from Caesalpinia sappan L. (Leguminosae) and elucidated the structure (see Table I). The structure of 2 (=4-hydroxyprotosapannin A) was further supported by comparison of the carbon signals in acetone-d$_6$ with those of 6 in the same solvent (Table I) by considering the hydroxylation shift of phenolic compounds. This is the second example of the isolation of compounds of this type. The name hematoxylol A is proposed for 2.

The molecular formula of the optically active compound 3' was determined by high resolution negative FAB-MS as C$_{20}$H$_{24}$O$_7$. The IR spectrum of 3' indicated the absence of any carbonyl group and the $^1$H-NMR spectrum of 3' in CDCl$_3$ showed signals due to four methoxyl groups at $\delta$ 3.88, 3.90, 3.91 and 3.93 (each 3H, s). For $^{13}$C-NMR comparison, a tetramethyl ether (7) was prepared from 2 after ketalization of the carbonyl group to protect it against methylation with diazomethane. The carbon signals of 7 due to C-1, 2, 3, 4, 10, 11, 12, 12a and 12b were observed at similar positions to those of 3' (Table II), indicating the presence of the same biphenyl moiety as that of 7 in 3'. This was supported by the aromatic proton signals of 3' at $\delta$ 6.79 (1H, d, $J = 6.6$ Hz), 7.01 (1H, d, $J = 8.6$ Hz), 6.81 (1H, s) and 6.83 (1H, s) in CDCl$_3$. The $^{13}$C-NMR spectrum of 3' in CDCl$_3$ exhibited two signals due to a O-CH$_2$C group at $\delta$ 67.1 and 80.8, a signal due to a C-CH$_2$C group at $\delta$ 38.9 and a signal due to a tetra-substituted carbon having an oxygen function at $\delta$ 71.8 (Table II). The $^1$H-NMR spectrum of 3' showed signals due to two O-CH$_2$ groups as two pairs of doublets at $\delta$ 3.86, 4.41 (each 1H, d, $J = 12.6$ Hz) and 3.46, 3.56 (each 1H, d, $J = 11.8$ Hz), together with signals due to a phenyl-CH$_2$C group at $\delta$ 2.59, 2.83 (each 1H, d, $J = 13.9$ Hz).
$^{13}$C-$^{1}$H COSY and $^{13}$C-$^{1}$H long range COSY spectra of 3' demonstrated the long range coupling of the tetra-substituted carbon signal with the methylene proton signals at $\delta$ 2.59, 2.83, 3.86 and 4.41 (Table II). It follows that 3' can be formulated as 7-hydroxy-3,4,10,11-tetramethoxy-7,8-dihydro-6H-dibenzo[b,d]oxocin-7-methanol (Table II). Since the $^{1}$H-NMR spectrum of 3 showed no methoxy proton signal, the name tetra-O-methylhetametoxyyl B is proposed for 3'. The absolute configuration at C-7 of 3' has not been assigned as yet. Nagai and Nagumo$^{4}$ isolated 3,7,10,11-tetrahydroxy-7,8-dihydro-6H-dibenzo[b,d]oxocin-7-methanol, named protosappanin B (8), from Caesalpinia sappan L. Subsequently, Saitoh et al.$^{40}$ isolated 10-O-methylprotosappanin B from the same plant. A comparison of the $^{13}$C-NMR spectrum of 3' with that of the trimethyl ether (9) of protosappanin B$^{3}$ supported the formulation of 3'. The biogenic correlation of I, protosappanins and related compounds with chamone derivatives has been proposed.$^{3-7}$

**Experimental**

Melting points were measured on a micro hot-stage and are uncorrected. NMR spectra were recorded on a JEOL JNM-GX 400 spectrometer in DMSO-$d_6$ at 50°C unless otherwise stated. FAB-MS were taken on a JEOL JMS-SX 102 spectrometer. Conditions of HPLC: column, TSKgel ODS-120T (21.5 mm i.d. x 30 cm); flow rate of mobile phase, 6 ml/min; detection, UV 254 nm.

**Extraction and Separation**

The powdered heartwood (125 g) collected in Mexico was extracted with hot MeOH. An aqueous suspension of the extract was chromatographed on Diaion HP-20 and eluted with H$_2$O, 30% EtOH, 50% EtOH, EtOH and acetone, successively. The 30% EtOH eluate, which tastes sweet, was chromatographed on silica gel. Elution with CHCl$_3$-MeOH-H$_2$O (40:10:1) provided three fractions, frs. 1–3.

The sweet fraction, fr. 2, was subjected to repeated chromatography on silica gel with C$_6$H$_6$-EtOAc-MeOH (5:10:1) followed by recrystallization from H$_2$O in a dark-colored flask under Ar gas to afford I (80 mg). I: sweet yellow prisms, mp 148–151°C. [x]$_D$$^{22}$ +90.3° (c=1.04, MeOH). FAB-MS m/z: 331 [M+Na]$^+$.

**References**


