Characterization and Reactions of a *Salix* Extractive with a Unique Ring System

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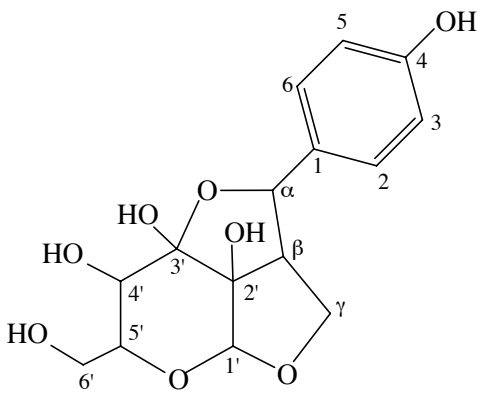
ABSTRACT

An extractive compound with a novel ring system was isolated from the wood of *Salix alba* and was given the name Salucci. The compound was primarily identified from the 1D NMR, 2D NMR and LC-MSMS data and the structure investigated through reactions and derivatizations.

INTRODUCTION

The fractions extractable from wood with organic solvents are generally very complex mixtures of numerous compounds of various types, none of which amounts to much more than 5% of the total fraction. Upon examination of the acetone extract of *Salix* species we have made the unanticipated discovery of the presence of a single compound that accounts for about 60% of the total extractive content and from 5-6% of the weight of the air-dried wood. Furthermore, upon extraction of the knotty part of the wood, up to 95% of the acetone extractives consisted of this single compound. NMR examination of the compound was not consistent with any of the *Salix* extractives reported in the literature. Furthermore, the compound appeared to have a fused ring system that has never been previously reported. Herein, we describe the structure elucidation by chemical derivitization and degradation studies and by NMR and mass spectrometry of the compound we call "Salucci", structure **I**.



I

RESULTS AND DISCUSSION

Sources

Five sources of *Salix* wood were examined over the course of the study. The original source of the acetone extractives came from a previous study of hybrid *Salix* (willow) clones [1] from Yugoslavia. These white willow clones varied in age from 1 to 14 years. Ground wood was acetone/water extracted prior to ball milling and the extractives were briefly examined by NMR. It appeared that a good proportion of the acetone extractives was a single compound and the data were inconsistent with extractives of *Salix* and close relatives which had been previously reported in the literature [2].

Two other sources of *Salix* were obtained. Two samples of weeping willow wood (*Salix babylonica*) came from the area in and around Madison, Wisconsin and two samples of willow, one weeping and one tortured willow (*Salix matsudana*) came from the Rotorua area of New Zealand. The amounts of Salucci isolated from these woods varied considerably and were partly dependent on the morphology of the wood. Higher concentrations of Salucci could be found in knotty tissue and tissue that was discolored compared to the "white" wood. Some samples of *Salix* bark were also examined to determine if Salucci was present in the bark extractives as well. In Table **1** is a summary of Salucci content in a variety of materials.

Source	sample	% wt dry material
Verona Weeping Willow	clear	1.7
	knotty	6.0
	bark	0.1
Yugoslav White Willow	combined	4.0
NZ Weeping willow	knotty	trace
NZ Tortured Willow	clear	0
	knotty	trace
Commercial White Willow	bark	trace

Table 1. Salucci content from various sources.

Initial extractions of the wood were done with 9:1 acetone/water mixtures yielding a mixture of extractive components of which Salucci was often predominant. To produce a cleaner source of the target compound the ground wood samples were first extracted with methylene chloride which gave on average about 3% dry wood weight yield of an extractive mixture. Chemical shift signals attributed to Salucci could not be found in the ^{13}C NMR of the methylene chloride extractives. Subsequent soxhlet extraction with dry acetone produced a concentrated source of Salucci which in some cases was nearly 95% Salucci and almost 7% yield based on the dry wood weight. Figure **1** shows the ^{13}C NMR spectrum for a typical methylene chloride extraction mixture and a spectrum from a subsequent acetone extraction mixture.

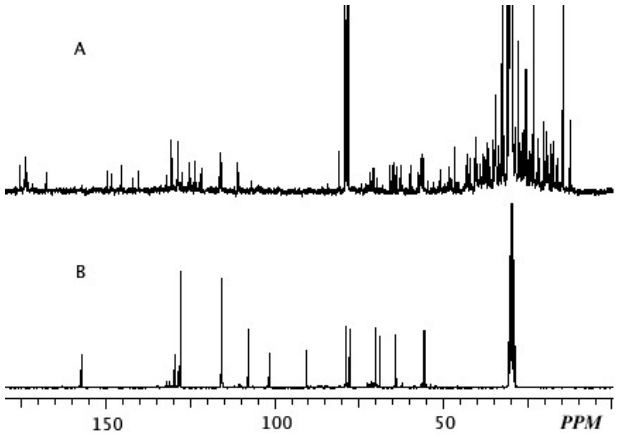


Figure 1. ^{13} C NMR spectra of methylene chloride (A) and acetone (B) extractives.

Characterization of Salucci

^{1}H and ^{13}C NMR indicated the formula $C_{15}H_{18}O_8$ which is consistent with structure **I** and a product formed perhaps from coumaryl alcohol and glucose. The ^{1}H, DEPT and ^{13}C NMR spectra are shown in Figure **2** (a,b, and c). The simplicity of the ^{1}H and ^{13}C spectra indicate a single isomer even though six chiral centers exist at 2', 3', 4', 5', α and β. Subsequently Salucci (Structure **I)** was shown to have optical rotation

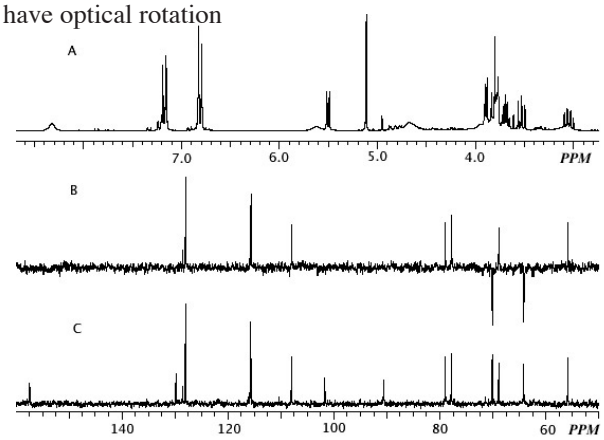
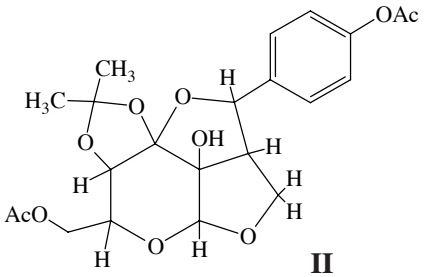


Figure 2. NMR spectra of Salucci

An attempt to improve the signal dispersion was successful by preparing an acetylated acetonide derivative of Salucci. Reaction with acetone, copper sulfate (catalyzed by toluenesulfonic acid) followed by treatment with acetic anhydride/pyridine gave a pure product in high yield (80%) which was consistent with structure **II**.



II

The ^{1}H, DEPT and ^{13}C NMR spectra are illustrated in Figure **3** (a,b and c). Clearly the ^{1}H spectrum appears to be 1st –order and remains consistent with the proposed structure **I**.

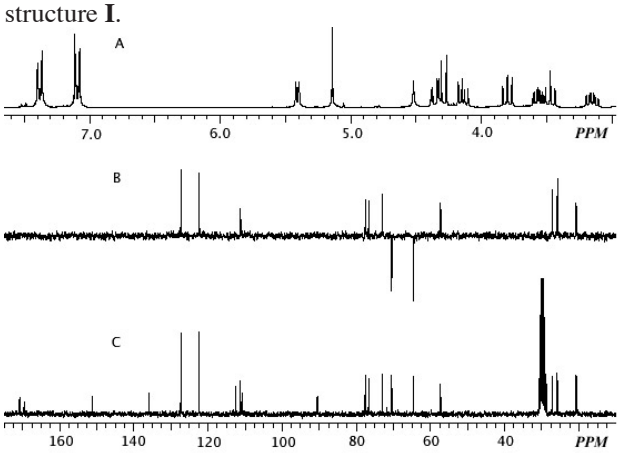


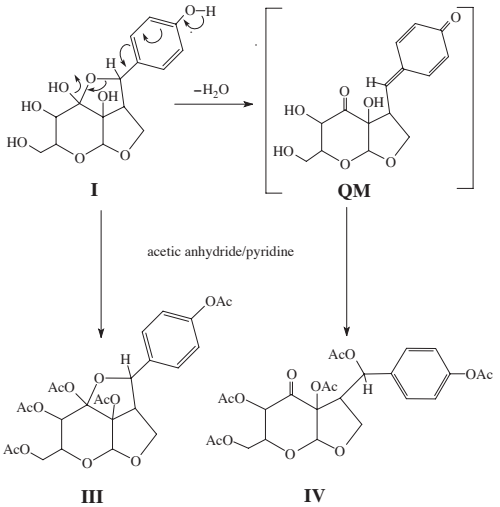
Figure 3. NMR spectra of the Salucci acetonide.

Reactions of Salucci

Derivitizations

Acetylation of Salucci gave a number of products depending on the conditions chosen. The only mono-acetate derivative of Salucci isolated consisted of the phenolic acetate. Two compounds were isolated with two acetate groups, the 4',6' di-acetate and the 4,6' di-acetate. One tri-acetate (4,4',6') and one tetra-acetate (4,3',4',6') were also isolated.

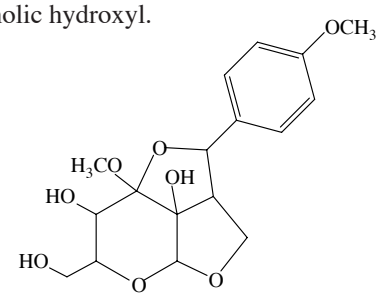
An interesting case was found when attempts to fully acetylate Salucci were made. The penta-acetate compound (**III**) was isolated along with an open version (**IV**) whereby the alpha-O-3' ether had been cleaved. This compound could be explained by quinone methide (**QM**) formation with subsequent loss of a water molecule followed by attack of acetate on the quinone methide as illustrated in Scheme **1**.



Scheme 1

Methylation

Methylation of Salucci gave the un-expected 3',4 di-methoxy compound (**V**). Attempts to prepare mono-methylated (phenolic) Salucci were unsuccessful which indicated that the 3'-hydroxyl had a comparable reactivity to the phenolic hydroxyl.



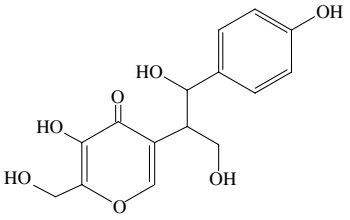
V

Acid Treatment

Heating a solution of Salucci in glacial acetic acid resulted in a mixture of 8 or more similar isomers which indicated a scrambling at some of the chiral centers.

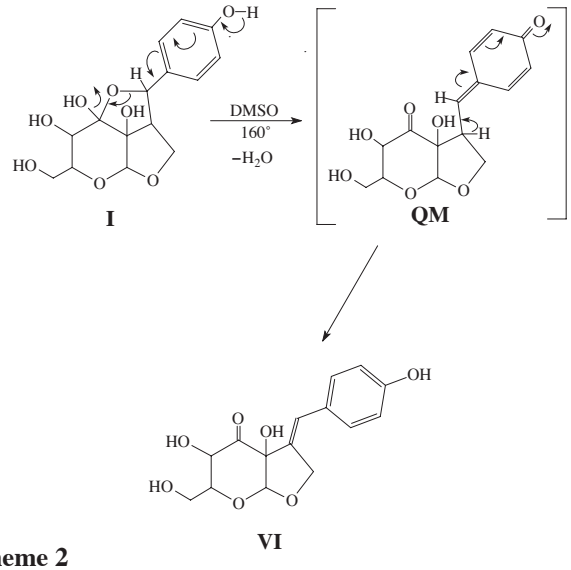
Alkaline Treatment

Heating a solution of Salucci in sodium hydroxide (1 M) resulted in one identifiable product (**VI**), although no credible mechanism could be found for its formation. NMR studies indicated the cleavage of the alpha and gamma ether bonds and the formation of a pyrone ring.



VI

Dehydration

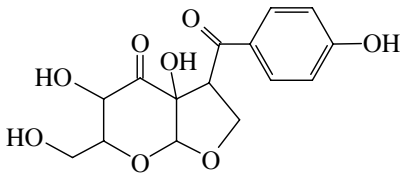


Scheme 2

Dehydration of Salucci was accomplished by heating a solution in dimethyl sulfoxide (DMSO) at 160˚C For several hours. NMR and MS analysis was consistent with structure **VII**, in Scheme **2**, which involved formation of the quinone methide, followed by a loss of the beta proton to regenerate the aromatic structure.

Oxidation

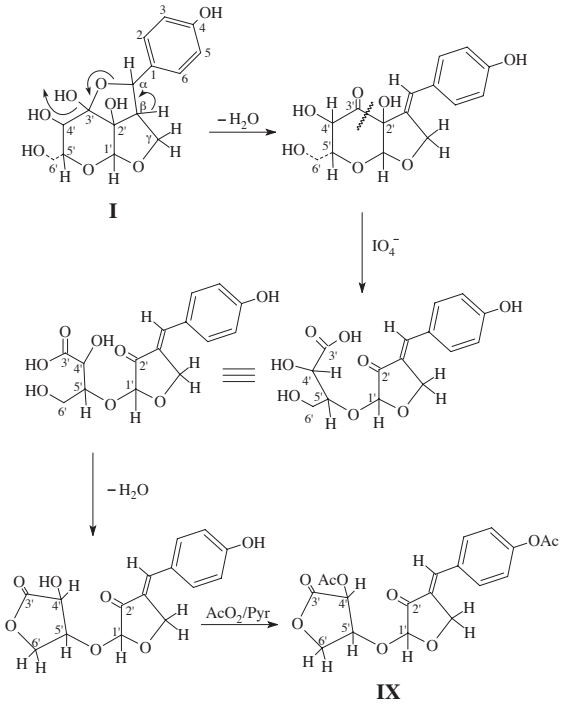
Oxidation of Salucci with dichlorodicyanobenzo-quinone (DDQ) in acetone gave a single compound in high yield. NMR analysis indicated structure **VIII**.



VIII

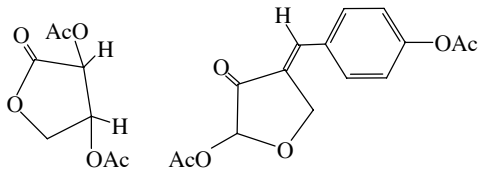
Oxidation

Oxidation of Salucci with sodium periodate in acetone generated an unstable intermediate of unknown structure, which when acetylated with acetic anhydride/pyridine gave a compound with tentative structure **IX**, in Scheme **3** which also illustrates a plausible mechanism for the oxidation.



Scheme 3

If the intermediate product from the periodate reaction was heated in DMSO at 160˚C the complex acetylated reaction mixture contained products **X** and **XI**.



X **XI**

Structure **X** was shown to be identical to an authentic sample of acetylated erythronic acid-γ-lactone, thus confirming half of the proposed structure **IX**.

Mass spectrometry

By ESI positive acquisition mode the sodium adduct of the quasi-molecular ion $[M+23]^+$ at *m/z* 349 was observed in very high abundance and its dimer $[M+23+M]^+$ was observed at *m/z* 675. In negative ion mode $[M-1]^-$ was observed at *m/z* 325.

The sample was diluted to reduce gas phase dimmer ions and 0.1% acetic acid was added to decrease sodium adduct abundance. The [M+1-18]+ ion at *m/z* 309 was observed and trapped for MS^n lineage analysis.

MS^2(309) exhibited fragments indicative of water , water and ethene or ethanol: 309 → 291, 273, 227, 191

MS^3(309,291) indicates losses of water and carbon monoxide or ethane:
309 → 291 → 263, 245, 227, 191

The acetonide derivative also exhibited the sodium adduct of the quasi-molecular ion $[M+23]^+$ at m/z =389 as its base peak.

Experimental

Isolation of Salucci (**I**): **I** (90-95%) pure could be obtained by pre-extracting air-dried sawdust with CH_2Cl_2 to remove the bulk of extractives, followed by extraction with dry acetone. A yield of 4-7% was obtained from knotty or discolored wood.

Preparation of Salucci acetonide (**II**): A mixture of **I** (412 mg, 1.26 mmol), anhy. $CuSO_4$ (320 mg, 2.00 mmol), toluenesulfonic acid monohydrate (52 mg, O.27 mmol), and dry acetone (25 ml) was stirred at room temp. for 19 hr. Powd. anhy. K_2CO_3 (412 mg, 3.00 mmol) was then added and the suspension was stirred for an additional 2 hr at room temp. Filtration of the suspension gave an amber filtrate, which when evaporated under vacuum left a foam (367 mg, 1.00 mmol, 79% yield), which was shown by NMR to be 95+% pure.

Acetylation of Salucci: (**III**, **IV**): Generally, acetylations were done with a 1/1 mixture of acetic anhydride/pyridine for several hours. Usually, mixtures of products were obtained which were chromatographed on a polystyrene gel column (BioRad biobeads S-X2). The open form (**IV**) of the penta-acetate was found only when a catalytic amount of dimetylaminopyridine was added to the acetylation mixture.

Acid treatment of Salucci: **I** (85 mg) was dissolved in deuterated acetic acid (0.5 ml) and the dark amber turbid solution was filtered through a plug of glass filter paper into an NMR tube. The clear amber solution was examined by NMR to confirm that there no reaction took place. The NMR tube was then heated in a steam bath for 3.5 hrs. Water (0.1 ml) was added and steam treatment was continued for 6 hrs. The NMR spectra of the dark red solution indicated about 8 isomers with very similar chemical shifts.

Alkaline treatment of Salucci: A solution of **I** (30 mg, 0.09 mmol) in 1 M NaOH (1 ml, 1.0 mmol) was gently heated in a steam bath for 15 min. The alkaline solution was acidified (pH=4) with Dowex 50W-X12 resin, filtered, and the soln. was evaporated under vacuum, leaving the product **VII** (20 mg, 0.06 mmol, 67%).

Dehydration of Salucci (**VI**): A solution of **I** (326 mg, 1.00 mmole) in dimethylsulfoxide (dmso) (2.0 ml) was heated in a 180 deg oil bath, under a slow nitrogen stream, for 4 hrs. The dark oil was then concentrated under vacuum (60 deg) for several hours to remove most of the dmso. Acetylation of the residue and separation on Bio-gel S-X2 gave a crude product **VI** (190 mg, O.40 mmol, 40% yield).

Oxidation of Salucci with DDQ: A solution of **I** (32 mg, 0.10 mmol) in deuterated(d_6) acetone (0.25 ml) was mixed with a solution of DDQ (25 mg, 0.11 mmol) in d_6-acetone (0.25 ml). After about 30 min. the brown soln was examined by NMR. The only products detected were **VIII** and the hydroquinone derived from DDQ.

Oxidation of Salucci with periodate: To a solution of **I** (326 mg, 1.00 mmol) in d6-acetone (1.5 ml) was added sodium periodate (219 mg, 1.02 mmol). The suspension was stirred in a small vial for 4 hrs. Filtration of the suspension gave a dark red filtrate, which was examined by NMR. No structure was elucidated from the spectra because it appeared to change with time. Acetylation of a portion of the product and separation on Bio-gel S-X2 gave the product **IX**.

Reaction of intermediate periodate product with dmso: A solution of the intermediate product in dmso was heated at 160 deg for 30 min. in an NMR tube (oil bath). Most of the dmso was removed under vacuum at 60 deg and the residue was acetylated as usual and the product mixture was applied to a Bio-Rad S-X2 column. The only products identified were **X** and **XI**.

Liquid Chromatography-Mass Spectrometry:

The LC-MS system was an Agilent 1100 MSD trap with a binary pump and an ESI-LC-MS interface. A Restek IBD column (50 x 2.1 mm, 5 um) with isocratic methanol:water (80:20) at 200 ul/min flow was used for chromatographic analysis. For directly infused samples the solvent was methanol:water (50: 50) at a flow of 5 ul/min. For infusion conditions were: 350°C dry gas, capillary voltage 3k-3.5k, nebulizer pressure 12-15 psi and dry gas flow 4 l/min. Capillary exit, trap drive, skimmer and octopole voltages were optimized on the target ions using segmented analyses.

REFERENCES

1. LL..Landucci, G.C. Deka, D.N. Roy, A 13C NMR Study of Milled Wood Lignins from Hybrid *Salix* Clones, Holzforschung **46**, 505-511 (1992).
2. J.W. Rowe, A.H. Conner, Extractives in Eastern Hardwoods – A Review (Forest Products Laboratory, Forest Service, USDA Madison, WI, General Technical Report FPL 18 , 1979).

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