BIOLOGICAL AND PHYTOCHEMICAL INVESTIGATION OF PLANTS XV.[1] *PTERYXIA TEREBINTHINA* VAR. *TEREBINTHINA* (UMBELLIFERAE)

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ABSTRACT.—Aqueous extracts of *Pteryxia terebinthina* var. *terebinthina* were shown to possess antiarrhythmic activity. A novel furanocoumarin, pterybinthinone, the known coumarins pteryxin and umbelliferone, and sucrose were isolated from this plant. Attempts to re-isolate a structurally undefined, defibrillatory substance by means of a published protocol were unsuccessful.

In 1958 Call and Fischer (1) initiated a phytochemical and pharmacological investigation of the roots of *Pteryxia terebinthina* var. *terebinthina* (Hook.) Coult. and Rose (Umbelliferae), which were collected near the Columbia River in Sherman County, Oregon. It was shown that within three weeks a diet containing 10 or 15% of this plant produced mortality rates of 33 and 50%, respectively, in groups of mice. Blood coagulation times in mice were increased in groups feeding on 5, 10 or 15% of the plant material in their daily ration. A hormonal effect due to the plant material was suggested, but not experimentally determined.

An uncharacterized compound was isolated and named pteryxin, which was claimed to be moderately piscicidal and to have *in vitro* spasmolytic activity twice that of khellin and four times that of papaverine on spontaneously contracting rat uteri (1). Details for the isolation of pteryxin were not given.

In 1962, Willette and Soine (2) investigated the roots of *P. terebinthina* var. *terebinthina* collected near Rufus, Oregon, reisolated pteryxin, and determined its structure. The mp reported for this substance was 81.5–82.5°; Call and Fisher (1) had previously recorded 81° as the mp for their pteryxin. In a subsequent study, Neilsen and Soine (3) reported the isolation of pteryxin from the roots of *Pteryxia terebinthina* var. *californica* (Coult. and Rose.) Mathias collected in Alpine County California.

In 1962 Bryan (4) reported the isolation of a water-soluble crystalline substance[3] having mp 191–192° from the roots of *P. terebinthina* var. *terebinthina* collected in the vicinity of Moses Lake, Washington. No other identifying features for the isolate were given, but it was stated that the substance dissolved in acetone with the aid of a small amount of water and that an aqueous solution of the isolate gave a weak blue fluorescence unaltered by acid or base. Bryan gave very detailed instructions for the isolation of this substance, which was obtained in a yield of 0.06% (4).

Bryan (4) reported that the isolate was essentially free from contractile depressant effects and that it was about seven times more potent than quinidine sulfate as a defibrillatory agent using the isolated rat heart model.

Prior phytochemical studies on *Pteryxia terebinthina* include the isolation of pteryxin, isopteryxin, osthole, anomalin and calipteryxin from the var. *californica*

[1]For the previous paper in this series see Benoit, P. S., H. H. S. Fong, G. H. Svoboda and N. R. Farnsworth, *Lloydia* 39, 160 (1976).

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[3]To be referred to subsequently as "Bryan's defibrillatory substance".

(3) as well as pteryxin (1, 2) and an uncharacterized water-soluble crystalline substance, mp 191–192°, from the var. *terebinthina* (4).

Pteryxin has been reported to have moderate piscicidal (1), *in vitro* uterine relaxant (1), vasodilating (5, 6), and hypotensive (7) effects; to decrease the frequency of heart contractions (5); and to inhibit acetylcholine, barium chloride- and serotonin-induced spasms of isolated rats and rabbit intestine (7–9). The spasmolytic structure-activity relationships of pteryxin, relative to a series of natural and synthetic furano- and pyranocoumarins, have been reported by Russian workers (9).

Because of our interest in biologically active plant constituents, a study was initiated to re-isolate "Bryan's Defibrillatory Substance", to determine its identity or structure, and to study its pharmacology in greater detail.

EXPERIMENTAL[4]

PLANT MATERIAL.—Four different plant collections were made and are identified as follows: *Pteryxia terebinthina* var. *terebinthina* (Hook.) Coult. & Rose, roots collected in the mountains of the Pacific Northwest (SP–1887); *Pteryxia terebinthina* (Hook.) Coult. & Rose (syn. *Selinum terebinthinum* Hook.) stems and roots, collected in the state of Washington (SP–2676); *Pteryxia terebinthina* (Hook.) roots, collected in California (SP–2677); and *Pteryxia terebinthina* var. *californica* (Coult. & Rose) Mathias, roots collected in California (SP–1701).[5]

PREPARATION OF EXTRACTS FOR PHARMACOLOGICAL EVALUATION.—Samples SP–1701, SP–1887, SP–2676, and SP–2677 were worked up as follows. A sample was percolated with an ethanol-water (1:1) mixture, the ethanol was removed *in vacuo* and the aqueous extract was lyophilized. The resulting powder was partitioned between a mixture of chloroform and water (1:1). The chloroform extract was evaporated *in vacuo* to give a residue (fraction C), and the aqueous extract was lyophilized (fraction W). All extracts and/or pure compounds evaluated for pharmacological activity were dissolved in distilled water, ethanol-water or DMSO (dimethyl sulfoxide)-water mixture. Controls were established with the same solvent.

ISOLATION OF BRYAN'S DEFIBRILLATORY SUBSTANCE.—Plant samples SP–1887 (roots of *Pteryxia terebinthina* var. *terebinthina*) and SP–1701 (*P. terebinthina* var. *californica*) were subjected to Bryan's procedure (4) for the isolation of the defibrillatory substance. From sample SP–1887, a water-soluble crystalline compound was obtained in 0.2% yield having mp 181–182°. The compound was homogeneous according to several different thin-layer chromatographic solvent systems. Sample SP–1701 yielded an identical substance having mp 182–183° in 0.76% yield. Both substances were identical with a reference sample of sucrose (mp, ir, tlc), and a mixture mp of each isolate and reference sucrose was undepressed.

ISOLATION AND IDENTIFICATION OF PTERYXIN (1).—The methanol extract from a 3.15 kg sample of SP–1887 roots on concentration *in vacuo* deposited crystalline material which, on recrystallization from methanol, gave an additional quantity (0.09%) of sucrose. The mother liquor was concentrated to a syrup (700 g) and partitioned between equal volumes of chloroform and water. Exhaustive extraction of the aqueous phase with ethyl acetate gave, after removal of the solvent, 7.6 g of a crude organic fraction, which was subsequently subjected to

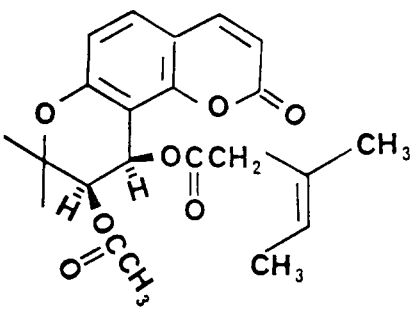
[4]Melting points were determined with a Kofler hot plate and are uncorrected. The uv spectra were obtained with a Beckman, model DB–6 grating spectrophotometer. The ir spectra were determined with a Beckman model 18–A spectrophotometer with polystyrene calibration at 1601 cm^{-1}. Absorption bands are recorded in wave numbers (cm^{-1}). PMR spectra were recorded in $CDCl_3$ or DMSO-d_6 solutions with a Varian model T–60A instrument, operating at 60 MHz with a Nicolet, model TT–7, Fourier Transform attachment. Tetramethylsilane was used as an internal standard and chemical shifts are reported in δ (ppm) units. Low resolution mass spectra were obtained with a Hitachi Perkin Elmer, Model RMU–6D, single-focusing spectrometer operating at 70 ev. High resolution mass spectra were obtained with a Varian 731 double-focusing spectrometer operating at 70 ev.

[5]Voucher specimens representing these collections were prepared and are on deposit in the Herbarium of the Department of Pharmacognosy and Pharmacology, College of Pharmacy, University of Illinois at the Medical Center, Chicago, Illinois 60612. All voucher specimens were examined by Dr. Robert E. Perdue, Jr., Medicinal Plant Resources Laboratory, Plant Genetics and Germplasm Institute, Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland. They were indistinguishable and could not be differentiated into varieties.

hplc[6] on silica gel PF_{254}[7]. The column was developed with a mixture of toluene-chloroform-acetone (53:25:22), and 20 ml fractions were collected. Work-up of combined fractions 21–39 from the column afforded pteryxin (**1**, 10 mg, 0.0003%) which crystallized from hexane-methanol and exhibited mp 78–81°. The physical data (uv, ir, proton nmr and mass spectral) were in agreement with those of pteryxin (**1**) (2, 10).

Thin layer chromatographic analysis [silica gel G, C_6H_6-$CHCl_3$-EtOAc (10:5:3)] indicated that the isolated pteryxin was contaminated with two minor impurities, and a reference sample was similarly contaminated. Further large scale purification was carried out by hplc[8] with Porasil A and elution with C_6H_6-$CHCl_3$-EtOAc (10:5:3) at a flow rate of 8 ml/min with recycling.

ISOLATION AND IDENTIFICATION OF UMBELLIFERONE (**2**).—Work-up of combined fractions 70–80 and treatment with methanol afforded crystals (18 mg, 0.00057%) having mp 205°. The compound was identical (tlc, ir, uv, proton nmr) with a reference sample of umbelliferone (**2**).



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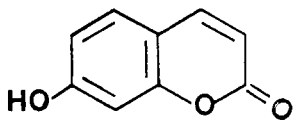

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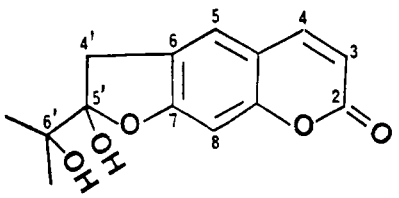

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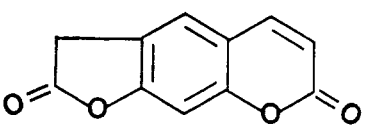

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ISOLATION AND STRUCTURE-ELUCIDATION OF PTERYBINTHINONE (**3**).—Work-up of combined fractions 101–105 yielded 35 mg (0.0014%) of a new furanocoumarin, pterybinthinone (**3**), mp 215° ir, ν max (KBr) 3340, 1710, 1620, 1572, 1275, 1262, 1190, 1145, 1043, 972, 860 and 825 cm^{-1}; pmr (d_6-DMSO) δ 1.26 (s, 6H, $-C(CH_3)_2$), 3.36 (bs, 2H, 2 x -OH, exchanged with D_2O), 3.94 (s, 2H, $-CH_2Ar$), 6.17 (d, 1H, J=9.5 Hz, 3-*H*), 6.70 (s, 1H, 8-*H*), 7.33 (s, 1H, 5-*H*), and 7.89 (d, 1H, J=9.5 Hz, 4-*H*), ms, *m/e* M^+ 262 (20%), 244 (2), 229 (6), 219 (6), 216 (4), 204 (25), 203 (11), 177 (13), 176 (100), 175 (40), 148 (10), 147 (15), 119 (3), 91 (8), 69 (13), 59 (76). Mass measurement: Found 262.0843, Calcd. for $C_{14}H_{14}O_5$ 262.0841.

Spectral data characterized pterybinthinone as a linear furanocoumarin in which the aromatic positions were not substituted and the furan ring was 4'5'-dihydro- and substituted at C-5'. Fourteen protons were observed in the pmr spectrum in agreement with the molecular formula $C_{14}H_{14}O_5$. Since two hydrogens were exchanged by D_2O, the two final oxygen atoms were present as hydroxy groups. The observation of singlets for the C-4' methylene at 3.94 ppm, and for a gem-dimethyl group at 1.26 ppm, limited these hydroxyl groups to C-5' and C-6'. Biosynthetic considerations suggest that pterybinthinone has structure **3**. In support of this assignment, pterybinthinone shows a characteristic loss of 58 mass units (m* at 158.8) and an intense fragment ion at *m/e* 59. This loss was followed by successive losses of 28, 29 and 28 mass units, each supported by a corresponding metastable ion.

[6]Chromatronix model 556 instrument.
[7]E. Merck, Darmstadt.
[8]Waters Associates model 6000 instrument.

The vicinal diol present in pterybinthinone was confirmed by treatment with periodic acid in aqueous dioxane at room temperature overnight. A single product was obtained, exhibiting a molecular ion at *m/e* 202, with important fragment ions at *m/e* 174 and 146 in agreement with structure 4 for this compound. Paucity of material prevented the acquisition of additional data.

IN VITRO ANTIARRHYTHMIC EVALUATION OF PTERYXIA TERBINTHINA EXTRACTS.—Isolated rat and rabbit hearts induced to arrhythmias with aconitine were used to evaluate extracts for antiarrrhythmic activity. Hearts were prepared in accordance with the technique described by Magnussen and Kudsk (11) and were perfused with Krebs-Hanseleit solution having the following composition (g/liter): NaCl 6.9, KCl 0.35, $CaCl_2$ 0.28, $NaHCO_3$ 2.1, $MgSO_4$-$7{\cdot}H_2O$ 0.11, KH_2PO_4 0.14 and glucose 2.0. Heart rate and rhythm were monitored from an electrogram and recorded.[9] Each isolated heart preparation was allowed to equilibrate for 30 min. before extracts were administered. Temperature of the perfusion medium was maintained at 37±1°. Cardiac arrhythmias were induced by introducing an aconitine solution (0.05 μg/ml) into the perfusate immediately prior to its entrance into the heart cannula. Cardiac abnormalities were noted as ventricular tachycardia and/or fibrillation. After an arrhythmic pattern was established for 10–20 min., the plant extract was injected into the perfusate and the effects were recorded. The results of these experiments are presented in table 1.

TABLE 1. *Effect of pteryxin and* Pteryxia terebinthina *extracts on aconitine-induced cardiac arrhythmias* in vitro.

Extract	Preparation					
	Rat heart (beats/min)			Rabbit heart (beats/min)		
	Control	Post Aconitine	Post Extract	Control	Post Aconitine	Post Extract
Pteryxin (W)[a, b]	(n=6) 155±26,	295±24	135±22	(n=8) 168±10,	403±28,	138±12
SP-2676(W)[c]	(n=5) 250±35,	5106±8,	220±33,	(n=6) 153±5,	320±71,	162±20
SP-2677(W)[c]	(n=5 230±36,	464±58	245±29	(n=9) 163±17,	357±43,	157±26

[a]W = Aqueous extract or solution.
[b]Dose = 0.2 mg/ml in a 50 ml organ bath.
[c]Dose = 2.0 mg/ml in a 50 ml organ bath.

IN VIVO ANTIARRHYTHMIC EVALUATION OF PTERYXIA TEREBINTHINA EXTRACTS.—Two methods were used for studying the *in vivo* antiarrhythmic effects of the various extracts. The first of these was by ouabain-induced cardiac arrhythmias in cats, followed by a determination of the effects of these extracts, as previously described (12). The second method involved aconitine-induced arrhythmias in cats, as described by Scherf (13). Results from the evaluation of the various extracts *in vivo* are presented in table 2.

TABLE 2. *Effects of* Pteryxia terebinthina *extracts on experimental cardiac arrhythmias* in vivo.

Extract	Heart rate (beats/min)			Arrhythmia		
	Control	Post Ouabain	Post Extract	Incidence	Number of Reversions	Percent Protected
SP-2675 (W)[a, b]	170±9,	190±13,	170±9	5/5	3/5	60%
SP-2677 (W)[b]	165±6,	155±16	177±29	4/4	0/4	0

[a]W = Aqueous extract.
[b]Dose = 47.6±5.6 mg/kg.

[9]Model 7B Grass polygraph.

EVALUATION OF PTERYXIN FOR ANTIARRHYTHMIC ACTIVITY.—Although pteryxin has been shown *in vivo* (5) and *in vitro* (6) to slow the heart, in addition to acting *in vivo* (7, 10) and *in vitro* (7) as a coronary vasodilator, no data have been published pertaining to the antiarrhythmic activity of this pyranocoumarin derivative. For this reason, pteryxin was evaluated for *in vitro* and *in vivo* antiarrhythmic activity by the methods previously described for crude extracts of *P. terebinthina*. The results are presented in tables 1 and 2.

DISCUSSION

Sucrose, pteryxin, umbelliferone, and a new furanocoumarin, pterybinthinone were isolated from the roots of *Pteryxia terebinthina* var. *terebinthina*. Pterybinthinone was determined to have structure **3** on the basis of interpretation of spectral data, and the results of a periodate oxidation experiment. Of these substances, only pteryxin had been previously isolated from this plant.

The main purpose of this study, however, was to reisolate and characterize Bryan's defibrillatory substance (4) and to further study the pharmacology of this substance, which Bryan reported to be seven times more active than quinidine as a defibrillatory agent (4).

Bryan (4) gave very explicit directions on the isolation procedure for his active substance. We followed these instructions using four different collections of *P. terebinthina*, and in all four cases isolated a water soluble crystalline material in yields ranging from 0.21–0.76%, with mp of 181–183°. However, Bryan's substance was reported in a yield of 0.06% with mp 191–192°. Further, Bryan claimed that his substance was very soluble in water with the solution exhibiting a weak blue florescence that was not apparently altered by the addition of dilute sulfuric acid or dilute ammonium hydroxide (4). These properties suggest that Bryan's substance was a coumarin glycoside.

We have unequivocally shown that the substance isolated from four different collections of *P. terebinthina* by Bryan's procedure is sucrose, which would not be expected to have defibrillatory activity.[10] Further, the crude fractions obtained in this study, failed to show antiarrhythmic activity of sufficient magnitude to lead one to believe that a defibrillatory substance seven times more active than quinidine could be present in *P. terebinthina*.

One can only speculate as to how Bryan could have determined that his isolated substance was such a potent defibrillatory agent. The most plausible explanation, although other possibilities exist, is that Bryan's substance was a mixture of sucrose plus one or more compounds having marked defibrillatory activity. Since we have shown that pteryxin has about one-half of the antiarrhythmic activity of quinidine, both *in vivo* and *in vitro*,[11] it does not appear that Bryan's defibrillatory substance could owe part or all of its activity to this compound. Further, pteryxin is only slightly soluble in water, and Bryan's active material was very soluble in water. Also, Bryan's defibrillatory substance has mp 191–192°, which precludes the possibility of its identity as sucrose (mp 181–183°), pteryxin (mp 78–81°), or a mixture of sucrose and pteryxin.[12]

One possible explanation for Bryan's success and our failure to isolate the active defibrillatory (antiarrhythmic) principle from *P. terebinthina* var. *terebinthina* is that of chemical variability between his and our plant materials. The

[10]This was experimentally shown by testing sucrose *in vivo* and *in vitro* by the methods described herein, with all results being negative.

[11]A detailed account of the defibrillatory activity of pteryxin will be subsequently published.

[12]Correspondence with Bryan revealed that his supply of defibrillatory substance was exhausted and thus no direct comparison could be made with any of our isolated compounds.

fact that an aqueous extract of our accession number SP–2676 afforded a 60% reversion of arrhythmias, whereas a similar extract prepared from accession number SP–2677 effected no reversions in cats (table 2), lends credence to this hypothesis.

The results summarized in table 1 indicate that aqueous extracts of *Pteryxia terebinthina* significantly decreased the aconitine-induced tachycardia ($p<0.05$) in perfused rabbit and rat hearts. However, as shown in table 2, only one of the extracts was effective in reversing ouabain-induced arrhythmias to normal rhythm in anesthetized cats. These observations suggest that aqueous extracts of *Pteryxia terebinthina* possess potential antiarrhythmic activity which is apparently effective *in vitro* and *in vivo*.

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