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Insecticides in Chinese Medicinal Plants: Survey Leading to Jacaranone, A Neurotoxicant and Glutathione-Reactive Quinol

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Sixty-two plant species from central China were purported to have insecticidal activity. Adult house fly (*Musca domestica*) toxicity assays were used to identify the two most active plants and guide the chromatographic isolation of the insecticidal components. The active ingredient of *Senecio palmatus* Pall. (Asteraceae) was characterized as methyl (1-hydroxy-4-oxocyclohexa-2,5-dien-1-yl)acetate (jacaranone) (**1**), previously known to have insect antifeedant activity. Mono- and bisglutathione (GSH) adducts are formed on incubation of **1** with GSH and rat liver GSH *S*-transferase. The toxic action of **1** in mice (intraperitoneal LD_{50} = 150−200 mg/kg) is associated with both neurological signs and GSH depletion in liver 90 min after treatment. *Paeonia suffruticosa* var. *papaveracea* (Andr.) Kerner (Paeoniaceae) was the other active plant found here to have 2′-hydroxy-4′-methoxyacetophenone (paeonol) (**2**) as an insecticidal ingredient in the root and in one case, for the whole plant, contaminated with *S*-*tert*-butylthiomethyl *O*,*O*-diethyl phosphorodithioate (terbufos) (**3**), a synthetic anticholinesterase insecticide.

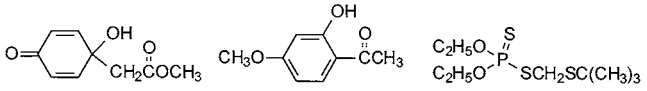
KEYWORDS: Glutathione conjugates; insecticide; jacaranone; paeonol; terbufos

INTRODUCTION

Plants have been a major source of chemical structure models for insecticides. Several important classes of synthetic insecticides have ancestors in nature (e.g., pyrethroids from the pyrethrins, methylcarbamates from physostigmine, and nereistoxin analogues from nereistoxin) or act the same way as natural products (neonicotinoids versus nicotine). New insecticidal plants are of continuing interest as a source of botanical insecticides and prototypes for synthesis and structure optimization, particularly when they represent a novel mode of action (*1*, *2*). Our search for new botanical insecticides was based on collecting 62 species used as medicinal plants or purported by local farmers or medical practitioners to have insecticidal activity. They all came from mountainous areas in central China. Bioassay-guided isolation led to the identification of jacaranone (**1**) from *Senecia palmatus* Pall. (Asteraceae) (*3*) and paeonol (**2**) and terbufos (**3**) from *Paeonia suffruticosa* var. *papaveracea* (Andr.) Kerner (Paeoniaceae) (*3*) (**Figure 1**) and information on their toxicology as indicated below.

MATERIALS AND METHODS

Plant Material. Whole plants of 62 different species (see the Supporting Information) were collected in mountainous areas of Lichuan County, Hubei Province of China, in August 2001. In addition *P. suffruticosa* was obtained as whole plants or roots from nearby Fang County in July 2000. These plants were identified by Professor Bingtao Li of the South China Agricultural University. The material was air-dried for up to 2 weeks at ambient laboratory temperature and several hours in a 50−60 °C incubator prior to pulverization. A methanol extract prepared by Soxhlet extraction for 24 h was concentrated in vacuo.



jacaranone (**1**) paeonol (**2**) terbufos (**3**)

Figure 1. Structures of two botanical insecticides [methyl (1-hydroxy-4-oxocyclohexa-2,5-dien-1-yl)acetate (jacaranone) (**1**) and 2′-hydroxy-4′-methoxyacetophenone (paeonol) (**2**)] and a commercial synthetic insecticide [*S*-*tert*-butylthiomethyl *O*,*O*-diethyl phosphorodithioate (terbufos) (**3**)] isolated in this study.

House Fly Bioassays. House fly (*Musca domestica*) adults (males and females) from a laboratory culture without any known insecticide resistance factors were used 3−7 days after emergence (*4*). The standard assay was made by placing granulated cane sugar (1 g) or a sugar cube (1.5 cm^3, 3.7 g) in a 20 mL vial followed by the test extract or fraction (10 mg) dissolved in acetone (0.5 mL). Acetone alone was used as a control. When the acetone was completely evaporated, leaving most of the dissolved compounds as a coating on the sugar, the house flies were introduced and the vial was closed with a screw cap. Mortality in these ingestion assays was recorded at 24 h. Alternative assays involved topical application or injection of the test solution (*4*) for isolation of the most potent insecticide (discussed last) and evaluation of biological activity, respectively. Each treatment was replicated 3 times, with 10 house flies per replicate.

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The insecticides isolated were those active in house fly bioassays. The first two compounds were of low potency compared with commercial insecticides and were active on ingestion but not topically. Their activity on other arthropods is not known except as specified below. The third compound was as potent as some of the best commercial products and was therefore isolated on the basis of topical assays.

Insecticidal Component Isolated from *S. palmatus.* The crude resin (16 g from 250 g of dried plant) was dissolved in methanol−water (4:1) (50 mL) and extracted with hexanes (250 mL × 5). A portion (5.5 g) of the aqueous methanol fraction (10 g after solvent evaporation) was chromatographed on a silica column (60 × 6 cm) with a gradient of methylene chloride−acetone (from 9:1 to 5:5). Eighty fractions were collected (20 mL each) and assayed. The first yellow fraction, eluting with methylene chloride−acetone (9:1), contained the insecticidal constituent. Evaporation gave a green gummy residue (110 mg), which was transferred to another silica gel column (65 × 2 cm) and eluted with methylene chloride−methanol (9:1) to give the pure insecticidal chemical (20 mg) as the second fraction. The structure was assigned as **1** by its identity to a synthetic standard (*5*, *6*) with respect to the ^{1}H and ^{13}C NMR spectra, UV spectrum, and GC/MS retention time and fragmentation pattern.

Reaction of 1 with Glutathione (GSH). The reaction products of **1** (11 mM) with GSH (11 mM) in 100 mM pH 7.4 phosphate buffer for 2 h at room temperature were analyzed by LC−MS on a model TSQ-70 instrument (Finnigan, San Jose, CA) with electron spray ionization (ESI) and a 250 mm × 2.1 mm i.d., 3 μm Supelcosil LC-18 column, developed with 20% water and 80% water−acetonitrile (9:1) with 0.1% trifluoroacetic acid at 0.2 mL/min. For in vitro studies on the enzyme-catalyzed reaction, the incubation mixture contained a 1.3 mM concentration each of GSH and compound **1** plus rat liver glutathione *S*-transferase (GST) (5−40 μg protein) (Sigma Chemical Co., St. Louis, MO) in pH 7.4 phosphate (10 mM) buffered saline (2.1 mL). The maximum decrease in the absorption spectrum was observed at 270 nm and attributed to reaction of **1** with GSH. Human placenta and equine liver GST (Sigma) were inactive.

In studies with male albino Swiss-Webster mice (25−30 g), **1** was administered intraperitoneally (ip) in Me_2SO (50 μL). The liver was removed at 90 min posttreatment from cervically dislocated mice and homogenized in phosphate-buffered saline as above at 4.5 mL/g. After deproteinization of the homogenate with an equal volume of 4% sulfosalicylic acid, the supernatant was analyzed for GSH as acid-soluble thiols using 5,5′-dithiobis(2-nitrobenzoic acid) (*7*, *8*). The GSH content of the controls averaged 2.2 mg/g of liver fresh weight.

Insecticidal Compounds Isolated from *P. suffruticosa.* Two batches of plant material from separate collections were examined, the first consisting of the root and the second of the whole plant. The sugar cube assay was used for the root extract and topical application for the whole plant extract. The root methanol extract (50 g from 830 g of initial dry weight) was subjected to the same isolation procedure as above prior to column chromatography on silica gel, first with hexanes−acetone (19:1), and then rechromatography with hexanes−methylene chloride (1:1), to give 148 mg of **2** as a white solid (178 ppm relative to the root dry weight). The whole plant extract, in contrast to the root extract, was surprisingly very potent as a topical toxicant to house flies. It was purified the same way by chromatography, first with hexanes−acetone (19:1), and then with hexanes−methylene chloride (1:1), to give **3** as a colorless liquid (29 ppm relative to the whole plant dry weight). Identifications were made by comparison to synthetic standards of **2** (Aldrich Chemical Co., Milwaukee, WI) and **3** (Chem Service, West Chester, PA).

RESULTS AND DISCUSSION

Survey of Plants Purported To Have Insecticidal Activity. Methanol extracts of the 62 plants were prepared at the South China Agricultural University and tested for toxicity to adult house flies at the University of California in Berkeley. The extracts of *S. palmatus* and *P. suffruticosa* were more toxic than those of 60 other species examined (see the Supporting Information). *S. palmatus* is used as an herbal medicine, considered to be a weed, and sometimes eaten as a vegetable. *P. suffruticosa* is cultivated for the beautiful flower, and the peeling from the root is one of the most important herbal medicines.

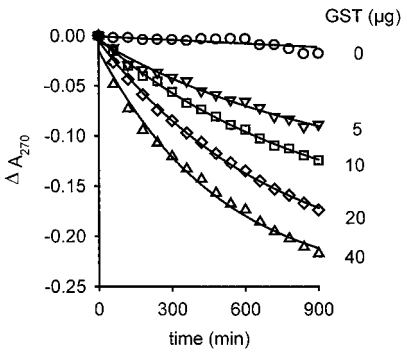

Figure 2. Reaction of **1** with GSH catalyzed by rat liver GST in pH 7.4 phosphate (10 mM) buffered saline.

Isolation and Identification of 1 as an Insecticidal Component of *S. palmatus.* *S. palmatus* gave the most insecticidal methanol extract except for that from *P. suffruticosa* whole plant considered later. Column chromatography of the *S. palmatus* extract on silica with methylene chloride−acetone and then methylene chloride−methanol gave a single compound as a pale yellow oil corresponding to 145 ppm relative to the whole plant dry weight. The starting crude resin added to granulated sugar for ingestion gave complete mortality of house flies at 10 mg and the final active ingredient at 10 μg with dose-dependent partial mortality at 3 and 1 μg. The resin and active ingredient act slowly over several hours. The insecticidal component was identified as **1** (**Figure 1**) by spectroscopy and synthesis (*5*, *6*).

Distribution and Biological Activity of 1. 1 is known to occur in Asteraceae (*Senecio* spp., *Emilia coccinea*, and *Pseudogynoxys cunninghammii)*, Bignonaceae (*Jacaranda mimosifolia*), and red algae (*Delesseria sanguinea*) (*6*). **1** is active in several biological systems and moderately toxic to many organisms. It is an antimicrobial (*6*, *9*) and antitumor agent (*10*, *11*). Although not detailed here, we found that **1** inhibited the root growth of germinating radish, spinach, and carrot at 1000 ppm. It is reported to be a growth inhibitor for tobacco cutworm (*Spodoptera litura*) larvae (*12*), to be toxic to brine shrimp (*Artemia salina*) (*13*), and to induce metamorphosis of marine invertebrate *Pecten maximus* larvae (*14*). Our further tests of **1** with house flies showed an LD_{50} of >500 μg/g topically and about 150 μg/g by injection with no synergism (*4*) by the mixed-function oxidase inhibitors piperonyl butoxide or *O*-propyl *O*-(2-propynyl) phenylphosphonate. The LD_{50} of **1** in mice treated ip was 150−200 mg/kg with neurotoxic poisoning signs (tail raising, tremors, lachrymation, and ataxia), leading to death in 30−90 min.

Reaction of 1 with GSH. The α,β-unsaturated quinoid structure of **1** suggested that it might react with GSH with possible catalysis by GST. On incubation of **1** with equimolar GSH and 5−40 μg of rat liver GST preparation, the absorbance at 270 nm decreased progressively with time and GST level (**Figure 2**) consistent with addition to the unsaturated quinol system. HPLC analysis established little reaction of **1** and GSH (1.0 equiv) in 10 mM phosphate (pH 7.4) buffered saline but extensive reaction in 100 mM pH 7.4 phosphate buffer. LC−MS revealed seven major peaks on monitoring A_{220} (**Figure 3**). Single ion monitoring (SIM) at *m*/*z* 490 appropriate for mono-GSH adducts gave four of the peaks. SIM at *m*/*z* 797 for

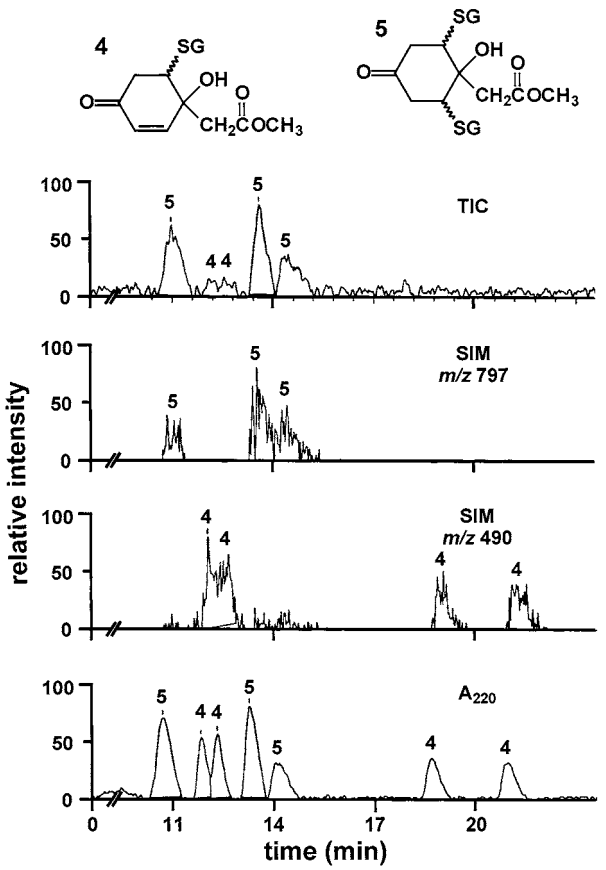

Figure 3. LC–MS analysis of four mono-GSH adducts (**4**) and three bis-GSH adducts (**5**) formed on reaction of **1** with 1 equiv of GSH in 100 mM pH 7.4 phosphate buffer. The normal delay is observed between the MS (top three panels) and A_{220} (bottom panel) peaks.

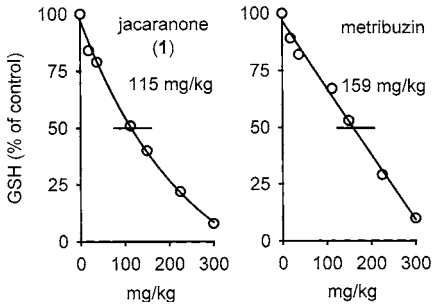

Figure 4. Effect of **1** and metribuzin on liver GSH level 90 min after treatment ($n = 3$). The dose for 50% reduction is given with the compound name.

bis-GSH adducts gave the remaining three. The total ion current (TIC) was more sensitive for the bis- than the monoadducts. These peaks are presumably the four diastereomeric monoadducts from Michael addition of the GSH thiol at the β position and three of the corresponding bisadducts. The same adducts are also formed but more rapidly on GST catalysis.

The possibility of GSH conjugation in vivo was examined with mice treated ip with **1**. The GSH content of liver decreased progressively with dose to the 50% level at 115 mg/kg compared with the herbicide metribuzin, a known liver GSH depletor (*7*), used as a positive control at 159 mg/kg (**Figure 4**). The reaction of **1** with GSH is probably a detoxification mechanism, perhaps protecting more critical target(s) in nerve from derivatization by **1**, but GSH depletion could also lead to oxidative tissue damage.

Isolation and Identification of 2 as an Insecticidal Component of *P. suffruticosa.* The methanol extract of *P. suffruticosa* root was active at 10 mg in the house fly sugar cube ingestion test with complete mortality within 1−2 h. Chromatographic isolation on silica with hexane−acetone and then hexane−methylene chloride gave a pure white solid, corresponding to 178 ppm relative to the root dry weight. The insecticidal ingredient was identified as **2**, previously known as a component of this species (*15*, *16*), by comparison with a synthetic standard. We found LD_{50} values for **2** in house flies of 1−3 mg/kg in the sugar cube assay and about 250 μg/g injected. It was not previously reported to have insecticidal activity. There are two indications for cytochrome P450-dependent detoxification of **2** in house flies, i.e., rapid recovery from knockdown (KD) and synergism by P450 inhibitors. Thus, the KD_{50} for **2** in the granulated sugar ingestion assay was 200−400 μg/g at 2 h, with complete recovery for 400 μg/g at 24 h. The ingested potency of **2** was increased at least 2-fold by topically applied piperonyl butoxide or *O*-propyl *O*-(2-propynyl) phenylphosphonate (250 μg/g) as P450 inhibitors (*4*). Synthetic **2** gave a mouse ip LD_{50} of >1500 mg/kg. Previously known biological activities of **2** include diuretic, antiarrhythmic, and antihypertensive activities and alteration of rheological parameters of blood in rats, guinea pigs, or quail (*17*−*21*), antimutagenic and antimicrobial activities (*22*, *23*), and promotion of DNA adduct formation and *N*-acetyltransferase activity in tumor cells (*24*).

Isolation and Identification of 3 from *P. suffruticosa.* The whole plant sample of *P. suffruticosa* had high topical insecticidal activity not evident with the root sample or with pure compound **2**. The active agent of the whole plant was isolated by chromatography monitored using topical house fly bioassay and found to be similar in potency to many commercial insecticides ($LD_{50} = 0.3-3$ μg/g). It was considered to be an anticholinesterase on the basis of the poisoning signs in house flies and mice. Isolation of **3** at 29 ppm and its identification as the synthetic phosphorodithioate insecticide terbufos were consistent with its high toxicity and type of action (*25*, *26*). Terbufos (**3**), one of the most toxic organophosphorus insecticides to both insects and mammals (*25*), was a contaminant instead of a constituent in this batch of *P. suffruticosa* collected as the whole plant. The use of terbufos in China is now restricted to specific soil applications.

CONCLUDING REMARKS

Four aspects of this investigation warrant special comment. First, it is a classical exploration in a remote area with guidance from local farmers or medical practitioners leading to special interest in 62 plant species. Second, the insecticidal ingredients of the two most effective plants were isolated and identified with structure confirmation by comparison with synthetic standards. Although they are not new compounds, the plants are important as herbal medicines and are of interest as possible crop protectants. Third, initial steps were taken in defining the mode of action and metabolism of jacaranone. Finally, one of the compounds was a very toxic synthetic insecticide, pointing out the ease with which botanical insecticides and herbal medicines can be contaminated during production or harvest.

ABBREVIATIONS USED

ESI, electron spray ionization; GSH, glutathione; GST, glutathione *S*-transferase; ip, intraperitoneally; KD, knockdown; SIM, single ion monitoring; TIC, total ion current.

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Supporting Information Available: List of plant species studied, NMR data for **1**−**3**, and ESI-MS for **4** and **5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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