exposure (Wills, 1972) to P and its metabolites.

There are discrepancies in in vivo ChE inhibition values among different laboratories for measuring blood ChE in carbamate poisoning. These discrepancies are due to sample dilution, substrate concentration, long reaction time, and washing of packed RBC (Witter, 1963; Winteringham and Fowler, 1966; Disney, 1966; Chin and Sullivan, 1968). All these factors influence the dissociation rate of the carbamate–enzyme complex and therefore result in underestimation of the degree of carbamate inhibition. For minimization of these factors, particular attention was given to the Auto-Analyze methodology used for this study. This methodology requires no predilution of plasma or RBC, low substrate concentration (1.0 mM), short reaction time (5 min), and no washing of packed RBC.

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# Isolation and Redefinition of the Toxic Agent from Cocklebur (*Xanthium strumarium*)

The highly toxic agent responsible for the poisonous properties of cocklebur, a common weed, has been isolated and identified as carboxyatractyloside. The toxin was identified by spectroscopic and chemical comparisons with authentic carboxyatractyloside.

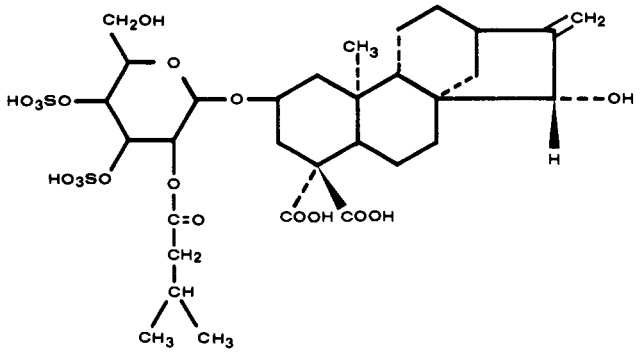
Cocklebur plants, *Xanthium spp.*, are widely distributed in the United States and are found in nearly all parts of the world. The plants have become especially noxious in South Africa and Australia. The poisonous properties of cocklebur are found only in seed and very young seedlings that still contain the cotyledon (cotyledon stage) (Marsh and Roe, 1924). Very young cocklebur seedlings, growing in pastures or fields, are particularly dangerous for swine in southern Georgia especially in the early spring. It was thought that the poisonous effects of cocklebur were largely due to the mechanical action of the burs (Hansen, 1920). More recently, Kuzel and Miller (1950) reported that the toxicity of the various species of *Xanthium* was due to hydroquinone present in the kernel and bur. We reexamined the etiologic agent in cocklebur as a result of an outbreak of cocklebur poisoning in South Georgia in the early spring of 1978. We were neither able to detect hydroquinone in cocklebur nor able to reproduce with it the characteristic lesions associated with cocklebur toxicity.

EXPERIMENTAL SECTION

**Plant Material.** Burs of *Xanthium spp.* were collected in fields in southern Georgia. Some burs were ground in a Wiley mill; others were germinated, and the very young seedlings (two-leaf stage) and older seedlings (four-leaf stage) were collected, lyophilized, and ground in a Wiley mill.

**Biological Assay.** The toxicity of crude extracts and other preparations was monitored with 30–40-lb shoats. The pigs were maintained on a complete 16% protein commercial swine ration and treated with an antihelmintic (Tramisol, Shell Chemical Co.). Initial crude preparations were given orally; purified toxin was administrated intravenously in sterile distilled water.

**Isolation Procedure.** Semiquantitative isolation of the potassium salt of carboxyatractyloside was performed by




first grinding either 100 g of whole burs or 33 g of freeze-dried seedlings in 1 L of acetone at high speed for 5 min. The slurry was filtered over two layers of cheesecloth, and the residue was returned to the blender and

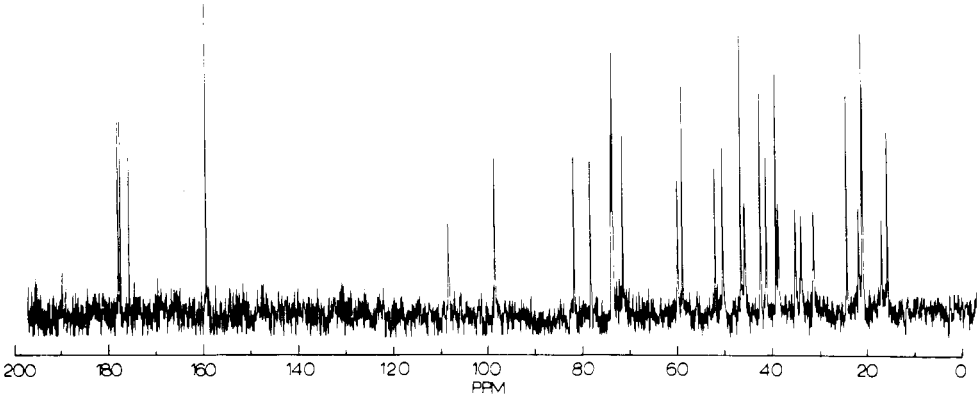



**Figure 1.** Proton decoupled $^{13}C$ NMR spectrum of carboxyatractyloside.

ground for 5 min with 500 mL of acetone–water (9:1) and again filtered. The bur residue was ground 2 more times with 500 mL of acetone–water. The crude extracts were vacuum filtered across glass fiber filter paper. The combined extracts were reduced in volume to 50 mL in a rotary evaporator at 40 °C and then partitioned twice against 100-mL portions of chloroform to remove lipophilic materials. The water layers were further reduced to 20 mL before adding 5.6 g of potassium chloride. The salt solutions were refrigerated overnight. The resulting precipitates were filtered off and treated with 100 mL of 85% ethanol and gentle heat (40 °C). The insolubles were filtered off and the combined ethanol solutions brought to semidryness on rotary evaporators at 40 °C. The precipitates were again treated with 85% ethanol and any remaining insolubles filtered off. The combined ethanol extracts were dried on a rotary evaporator and then vacuum dried overnight at 40 °C. Thin-layer chromatography (TLC) indicated that the final preparation was more than 95% pure. Carboxyatractyloside (potassium salt) was crystallized from 50% aqueous ethanol solution.

**Analytical Methods.** Commercial preparations of carboxyatractyloside (as potassium salt) were purchased from Sigma Chemical Co. Hydroquinone was purchased from Fisher Scientific Co.

Thin-layer chromatographic analysis of carboxyatractyloside and hydroquinone was accomplished with 0.5-mm layers of silica gel G-HR (Brinkmann Instruments Co.) developed in 1-butanol–acetic acid–ethyl ether–water (45:30:15:5 v/v/v/v) for carboxyatractyloside and toluene–ethyl acetate–formic acid (5:4:1 v/v/v) for hydroquinone. Both were detected by spraying plates with freshly prepared anisaldehyde spray (5 mL of anisaldehyde in 50 mL of glacial acetic and 1 mL of concentrated sulfuric acid) and heating them for 5–10 min at 120 °C (Stahl, 1965). The minimum quantity detectable by this system was ~1.5 μg of carboxyatractyloside. Analyses for hydroquinone were done according to the method of Kuzel and Miller (1950) and supplemented by TLC analyses.

Melting points were determined on a Kofler micromelting point apparatus and were uncorrected. Ultraviolet spectra were taken in distilled water with a Perkin-Elmer Model 552 UV–visible spectrophotometer. Infrared spectra were taken with a Perkin-Elmer Model 257 spectrophotometer. Samples were analyzed as a mull.

Fourier transform $^{13}C$ NMR spectra were obtained on a Varian Associates XL-100-12 NMR spectrometer equipped with the 620-L disk data system. The sample was prepared in $D_2O$ solution and run in a 5-mm tube. Chemical shifts were determined relative to an external dioxane reference and then converted to the $Me_4Si$ scale. Single-frequency, off-resonance proton decoupled (sford) $^{13}C$ spectra were obtained to determine the number of protons attached to each carbon.

**Table I. $^{13}C$ Chemical Shifts**

| | | |
|---|---|---|
| 17.34, q | 40.56, s | 74.67, d (2) |
| 18.56, t | 42.59, d | 74.96, d |
| 22.45, q | 43.85, t | 79.32, d |
| 22.63, q | 47.14, t | 82.79, d |
| 23.40, t | 47.96, s | 99.29, d |
| 25.82, d | 51.70, d | 109.07, t |
| 32.71, t | 53.31, d | 159.36, s |
| 35.33, t | 60.13, s | 175.36, s |
| 36.49, t | 61.12, t | 177.14, s |
| 40.12, t | 72.57, d | 177.62, s |

## RESULTS AND DISCUSSION

The toxin from cocklebur was isolated from burs and seedlings as a single spot at $R_f$ 0.6, mp 289–292 °C dec. All direct analytical comparisons (mp, TLC, IR, UV, and $^{13}C$ NMR spectral analyses) of the toxin from cocklebur with an authentic sample of potassium carboxyatractyloside confirmed the identity of the toxin as carboxyatractyloside. The proton decoupled $^{13}C$ NMR spectrum of carboxyatractyloside shows 30 peaks (Figure 1). Intensity considerations suggest that the peak at 74.67 ppm is due to two carbons. Chemical shifts are given in Table I along with the results of the sford spectrum. The amount of carboxyatractyloside as determined by gravimetric methods was 0.457% for burs and 0.12% for seedlings (two-leaf stage). Carboxyatractyloside was not detected in older seedlings (four-leaf stage). Danieli et al. (1972) first isolated the novel glycosides atractyloside and carboxyatractyloside from the rhizomes of *Atractylis gummifera* L. Subsequently, Kupiecki et al. (1974) isolated a hypoglycemic agent from cocklebur (*Xanthium strumarium*) that was later identified by Craig et al. (1976) as carboxyatractyloside. They did not associate the hypoglycemic properties of carboxyatractyloside with the specific diagnostic changes in swine caused by cocklebur poisoning. Kuzel and Miller (1950) reported that the toxic properties of the various species of *Xanthium* were due to hydroquinone. They reportedly reproduced the toxic effects of *Xanthium* poisoning found in the field with hydroquinone. Using the reported method of Kuzel and Miller (1950) followed by TLC analyses, we were unable to detect any hydroquinone in *X. strumarium*. Furthermore, we failed to reproduce the typical lesions of *Xanthium* toxicity after oral administration of authentic hydroquinone at levels up to twice the lethal dose (350 mg/kg) reported by Kuzel and Miller (1950). Conversely, the clinical signs and typical lesions (ascites and hepatic lesions) produced by administration of cocklebur seedlings and purified carboxyatractyloside were indistinguishable from each other and

from those observed in field cases. The details concerning the comparative pathology of poisoning by cocklebur seedings and authentic carboxyatractyloside in swine will be reported elsewhere (B. P. Stuart, R. J. Cole, and S. H. Gosser, unpublished data).

Carboxyatractyloside appeared to be located primarily in the cotyledon of the plant, and the concentration of carboxyatractyloside diminished rapidly after germination of the seed and the disappearance of the cotyledons. Metabolic activity in the cotyledon was directly associated with a concomitant disappearance of carboxyatractyloside and loss of toxicity. This rapid decrease in toxicity as the plant grows older no doubt has been the source of confusion by early investigators about the toxicity or lack of toxicity of the cocklebur plant.

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# Chemistry of Toxic Range Plants. Volatile Constituents of Broomweed (*Gutierrezia sarothrae*)

The major mono- and sesquiterpenes in the essential oil of *Gutierrezia sarothrae* have been identified by gas chromatography–mass spectrometry as α-pinene, myrcene, linalool, *cis*-verbenol, *trans*-verbenol, verbenone, geraniol, caryophyllene, and γ-humulene.

Broomweed, turpentineweed, or snakeweed (*Gutierrezia sarothrae*), a perennial resinous shrub widespread on dry rangeland from Texas to California and northward to Idaho, has been responsible for major economic losses due to abortion and death among range animals (Kingsbury, 1964). Cattle appear to be particularly susceptible to the abortifacient effects of broomweed, although sheep and goats are also affected to a lesser extent. Common features of broomweed-induced abortion in cattle are production of weak and underweight calves which frequently fail to survive, retained placenta, and hemorrhage. The plants appear to be most toxic in the early growth stages and are more toxic when growing on impoverished, sandy soils than on richer soils.

Intravenous injection of a saponin isolated from broomweed into pregnant rabbits, goats, and cows induced abortion and produced symptoms similar to those observed in cows poisoned by consuming the plant on the range. The saponin was shown to be abortifacient when administered orally to pregnant rabbits. However, both a saponin from lechugilla (*Agave lecheguilla*), known to be toxic, and a supposedly nontoxic, commercial pharmaceutical-grade saponin produced abortion and/or death when administered intravenously to pregnant rabbits (Dollahite et al., 1962; Shaver et al., 1964).

Since certain terpenes have been reported to stimulate the central nervous system and may also cause menorrhagic abortion (von Oettingen, 1963), knowledge of the composition of the volatile fraction of broomweed could provide information for comparison of the volatile terpene constituents with those of other abortifacient range plants.

The composition of the steam-volatile essential oil obtained from broomweed is reported herein.

## EXPERIMENTAL PROCEDURES

Leaves and small stems of *Gutierrezia sarothrae* (Pursh.) Britton et Rusby were collected from young plants in the preflower stage on April 5, 1979, near Eunice, NM. The material was air-dried and ground to pass through a 1-mm screen.

A sample of the ground plant material (100 g) in deionized, odor-free water (500 mL) was extracted with heptane (50 mL) containing Ionox 330 in a Likens-Nickerson apparatus held at 100-mm pressure (~50 °C water temperature) for 8 h. The heptane extract was concentrated by distillation of the solvent through a Vigreaux column, the concentrate weighed, and the percentage of heptane remaining determined by gas chromatography. The concentrated extract was then transferred to a small pear-shaped flask and the residual heptane distilled off through an air condenser.

Gas chromatography (GC) was carried out by using a Hewlett-Packard 5830 equipped with a 30 m × 0.25 mm i.d. glass SP2100 methyl silicone column and flame ionization detector. The column was programmed from 60 to 250 °C at 4 °C/min. Samples were injected with a split ratio of 1:50 at a linear velocity of 23.5 cm/s. Identification of components was based upon GC–MS data obtained with a quadrupole-type mass spectrometer (Electronic Associates Quad 300 mass filter; Finnigan Corp. 3000-1B