of *n*-propyltrichlorosilane and 546 g., 4 moles, of sulfuryl chloride activated by 1 g. of benzoyl peroxide. The flask was fitted with an efficient reflux condenser connected to a phosphorus pentoxide tube and the reaction mixture heated on the steam-bath. Reaction started immediately as evidenced by a vigorous evolution of hydrogen chloride and sulfur dioxide. The reaction mixture was refluxed for twelve hours, at the end of which time evolution of hydrogen chloride and sulfur dioxide had ceased. The product, 797 g., was then fractionated in a column of 30 theoretical plates. There were obtained: (I) crude α-chloro-*n*-propyltrichlorosilane, 110 g., b. p. 153–158°, which on refractionation gave 78 g. (0.37 mole) of pure α-chloro-*n*-propyltrichlorosilane, b. p. 157° (739 mm.); (II) β-chloro-*n*-propyltrichlorosilane, 273 g. (1.3 moles) b. p. 162° (729 mm.); and (III) γ-chloro-*n*-propyltrichlorosilane, 242 g. (1.15 moles), b. p. 178.5° (732 mm.). In addition, 180 g. (1 mole) of unreacted *n*-propyltrichlorosilane was obtained. The chloro-*n*-propyltrichlorosilanes were analyzed for chlorine content by peroxide fusion in a Parr bomb.

Anal. Calcd. for $C_3H_8SiCl_4$: Cl, 66.9. Found: for the α-chloro compound, 66.7; for the β-chloro compound, 67.0; for the γ-chloro compound, 67.0.

Alkali Titration of the Chloro-*n*-propyl-trichloro-silanes.—Weighed samples, about 0.7 g., were added to 20 cc. of methanol. Addition of distilled water, 50 cc., was followed by titration with 0.5 *N* sodium hydroxide using phenolphthalein.

Titration of α-chloro-*n*-propyltrichlorosilane gave: Cl, 50.1. Calcd. for Cl attached only to silicon, 50.2.

Titration of β-chloro-*n*-propyltrichlorosilane gave: Cl, 67.0. Calcd. *total Cl* for $C_3H_6SiCl_4$ is 66.9.

Titration of γ-chloro-*n*-propyltrichlorosilane gave: Cl, 50.2. Calcd. for Cl attached only to silicon, 50.2.

Relative Activity of C–Cl Bonds Alpha and Gamma to Silicon toward Alcoholic Alkali.—Weighed samples (0.5 g.) of I, III, α-chloroethyltrichlorosilane, and *n*-hexyl chloride were placed in 50-cc. flasks containing 18 cc. of 1.5 *N* ethanolic potassium hydroxide and the flasks were quickly stoppered for reaction at room temperature, or quickly fitted with reflux condensers if the reactants were to be refluxed. Half of these solutions were allowed to stand at room temperature for one hour. The others were refluxed for two hours. Extent of reaction was then determined by Volhard titration for chloride ion.

Acknowledgments.—Our thanks are due the Miner Laboratories and the Minnesota Mining and Manufacturing Company.

Summary

1. The three chloro-*n*-propyltrichlorosilanes have been synthesized by the peroxide-catalyzed chlorination of *n*-propyltrichlorosilane with sulfuryl chloride.

2. The gamma carbon in *n*-propyltrichlorosilane is somewhat less easily chlorinated than the beta carbon atom. The alpha carbon is chlorinated with relative difficulty due to the proximity of the $-SiCl_3$ group.

3. The reactivity of a C–Cl bond gamma to silicon is far greater than that of primary alkyl chlorides. At room temperature, it reacts completely with alcoholic alkali in one hour.

4. The beta C–Cl bond in β-chloro-*n*-propyltrichlorosilane reacts quantitatively on titration with 0.5 *N* alkali.

STATE COLLEGE, PENNA. RECEIVED NOVEMBER 5, 1945

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF WINTHROP CHEMICAL COMPANY, INC.]

Antibacterial Substances from *Asarum canadense*. I. Isolation, Physical Properties and Antibacterial Action

BY CHESTER J. CAVALLITO AND JOHN HAYS BAILEY

Although considerable chemical work has been done with constituents of species of *Asarum*,[1] it is only recently that screening tests for antibiotic activity in plants have shown that some members of this genus demonstrate weak activity against Gram positive bacteria.[2,3]

From *Asarum canadense* var. *reflexum* (Wild Ginger) there have been isolated two antibiotic substances, A, a very potent colorless compound of tentative empirical formula $C_{21}H_{20}O_8N_2S$, and B, a lemon-yellow acid of tentative formula $C_{16}H_{11}O_7N$, which shows considerably less activity. The activity has been observed against only Gram positive organisms. Each product has been obtained in a yield of approximately 20 milligrams per kilogram of fresh leaves and stems (moisture content about 85%).

Substances A and B are soluble in ethanol, acetone, chloroform, ethyl acetate and dioxane, almost insoluble in water, benzene and the Skellysolves; B forms water-soluble salts. Both compounds are readily inactivated by cysteine[3] and fall in line with the mode of action theories postulated for antibiotics.[4] The inactivation of A by cysteine shows similar time–concentration–*p*H effects as demonstrated for penicillin. Product A may be readily adsorbed from aqueous solutions by means of activated charcoal.

Product A is neutral in aqueous ethanol solutions and cannot be removed from a chloroform solution by extraction with aqueous sodium bicarbonate solution or by tenth normal hydrochloric acid. Treatment of A with alkalies (*p*H 10) and back titration with acid demonstrates the liberation of acidic groups and formation of a highly greenish-fluorescent product. Vapors from the hydrolysis solution darken lead acetate paper indicating formation of hydrogen sulfide.

Although pure B was prepared readily, A was

(1) Kaku, *et al.*, *C. A.*, **33**, 546 (1939); Nomura, *et al.*, *ibid.*, **24**, 2445 (1930); Takahashi, *ibid.*, **25**, 4975 (1931); Chou and Chu, *ibid.*, **30**, 241 (1936); Gerö, *ibid.*, **24**, 2235 (1930); Orient, *ibid.*, **28**, 6247 (1934); Bruckner and Szeki, *J. prakt. Chem.*, **134**, 107 (1932).

(2) Osborn, *Brit. J. Exp. Path.*, **24**, 227 (1943).

(3) Cavallito and Bailey, *Science*, **100**, 390 (1944).

(4) Cavallito, Bailey, Haskell, McCormick and Warner, *J. Bact.*, **50**, 61 (1945).

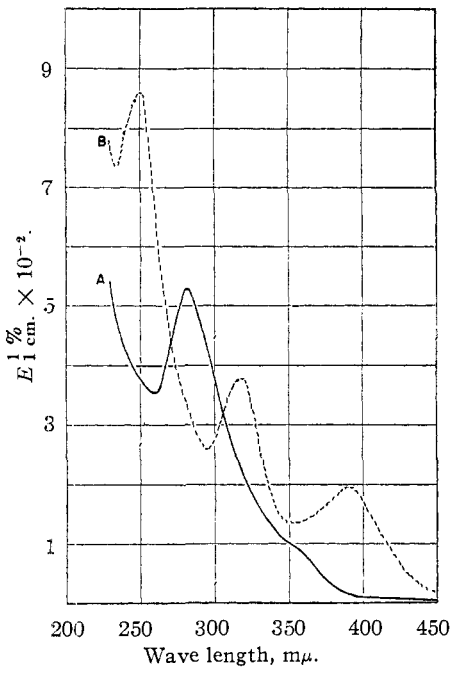


Fig. 1.—Absorption spectra of antibiotics A and B.

obtained in the pure state free of fluorescent impurities only at the expense of much lowered yields. Compounds A and B do not have sharp melting points; B darkens slowly between 230–260° without melting; A decomposes slowly above 160°, rapidly at 175°. Both compounds have characteristic ultraviolet absorption spectra (Fig. 1). The curves presented have been prepared by Dr. G. W. Ewing. The $E^{1\%}_{1\ cm.}$ values in U. S. P. ethanol were: for A, 529 at λ282; for B, 860 at λ250, 376 at λ318 and 194 at λ390.

Most of the antibacterial studies have been carried out with A, the results of which are presented in Table I. The activity of B against a few organisms is given in the experimental section.

Product A readily forms supersaturated solutions when an alcohol solution is diluted with water, particularly if A is not absolutely pure. A concentration of 0.06 mg. per milliliter produced a zone of inhibition against *Staphylococcus aureus* equivalent to one Oxford unit of penicillin G per milliliter when tested by the cylinder-plate assay method. This concentration is slightly greater than that of a saturated aqueous solution of A. By the same method, B as the sodium salt shows an inhibition equivalent to one unit of penicillin in a concentration of 0.25 mg. per milliliter. Compound A appears much more active than B when tested by the serial dilution method in which rate of diffusion in agar is not a factor.

Preliminary toxicity tests in mice of product A suspended in sesame oil are indefinite, deaths resulting usually in two to three days after intra-

TABLE I

ANTIBACTERIAL ACTION[a] OF A AGAINST BACTERIA

Organism	Milligrams per cubic centimeter of antibiotic in test											
	0.1	0.075	0.05	0.025	0.01	0.0075	0.005	0.0025	0.001	0.00075	0.0005	0.00025
B. subtilis C4	0	0	0	0	0	0[b]	0	±	+	+	+	+
B. subtilis S8	0	0	0	0	0	0	0	0	+	+	+	+
B. subtilis WIN	0	0	0	0	0	0	0	0	+	+	+	+
Staph. albus 151	0	0	0	0	0[b]	0	0	±	+	+	+	+
Staph. aureus SA10	0	0	0	0	0	0	0	±	+	+	+	+
Staph. aureus SA1	0	0	0	0	0	0	0	0	+	+	+	+
Staph. aureus 209[c]	0	0	0	0	0	0[b]	0	0	±	+	+	+
B. coli 71	±	+	+	+	+	+	+	+	+	+	+	+
B. paratyphosus A[d]	±	±	±	+	+	+	+	+	+	+	+	+
B. cereus 15	0	0	0	0	0	0	0	0	0[b]	0	0	±
B. mycoides	0	0	0	0	0	0	0	0	0[b]	0	+	+
Sarcina lutea	0	0	0	0	0	0	0	0	0	0	±	+
Strep. fecalis 10C 1[e]	0	0	0	0	0	0	±	+	+	+	+	+
Strep. hemolyticus C203	..	..	..	..	±	+	+	+	+	+	+	+
Strep. viridans	..	..	..	..	+	+	+	+	+	+	+	+
Pneumococcus Type I	..	..	..	..	0	+	+	+	+	+	+	+
Pneumococcus Type II	..	..	..	..	+	+	+	+	+	+	+	+
Pneumococcus Type III	..	..	..	..	±	+	+	+	+	+	+	+
Cl. botulinus A	0	0	0	0	0	0	0	0	0	±	+	+
Cl. botulinus B	0	0	0	0	0	0	0	0	0	0	±	+
Cl. botulinus C	0	0	0	0	0	0	0	0	0	0	0	±
Cl. botulinus E	0	0	0	0	0	0	0	0	±	+	+	+
Cl. histolyticus	0	0	0	0	0	0	0	±	+	+	+	+
Cl. tetani	0	0	0	0	0	0	0	0	0	0	±	±

[a] 0 is complete growth inhibition, ± is questionable, + is normal growth. [b] Lowest concentration showing no growth upon sub-culturing 0.1 cc. of culture into 5 cc. of fresh broth, *i. e.*, bactericidal level. [c] Penicillin sensitive Gram positive organism, growth completely inhibited by 3×10^{-5} mg. of penicillin G per cc. [d] Gram negative organism, growth completely inhibited by 0.01 mg. of penicillin per cc. [e] Penicillin resistant Gram positive organism, growth completely inhibited by 0.006 mg. of penicillin per cc.

peritoneal administration of quantities of the order of 5 milligrams per kilogram.

Experimental

Isolation of A and B.—Five kilograms of fresh leaves and stems were finely ground, added to 5 liters of 95% ethanol and stirred occasionally during one hour. The liquid was squeezed out through muslin and filtered (filtrate a). To the marc was added 4 liters of 95% ethanol and the mixture stirred occasionally during one hour. The liquid was squeezed out, 3 liters of water was added to precipitate chlorophyll and the mixture was filtered through Filter-Cel (filtrate b). Filtrates a and b were combined and the alcohol was distilled off by means of a water pump (20 mm.). (Product A, although relatively insoluble in water in the pure state, easily forms supersaturated solutions in the presence of impurities.) The aqueous residue in 2.5-liter aliquots was extracted twice with approximately 250 cc. of chloroform. The combined chloroform solution was extracted at least twice with one-third its volume of 1% sodium bicarbonate solution. The sodium bicarbonate extracts upon acidification yielded approximately 100 mg. of crude B as a yellow precipitate. The chloroform solution was evaporated under reduced pressure, the residue was extracted with 25 cc. of dioxane and filtered free of insoluble matter. To the dioxane solution was added 100 to 150 cc. of Skellysolve B and the turbid solution refrigerated for at least three hours, during which time crude A was precipitated.

Compound A was purified as follows: the crude material was dissolved in the least volume of warm 95% ethanol, filtered, diluted with five volumes of water and refrigerated overnight. Between 100 and 150 mg. of yellow-brown precipitate was obtained. This was again dissolved in a minimum of warm 95% ethanol, the solution shaken with a very small amount of charcoal (Darco G-60), filtered and refrigerated overnight. Spheroidal particles precipitated out slowly and often clung to the walls of the flask. The product was repeatedly purified by precipitation from alcohol until obtained colorless.

Crude B was dissolved in dilute alkali, treated with a small amount of charcoal, filtered and acidified. The precipitate was re-crystallized as yellow needles from hot 95% ethanol.

Anal.[5] Found for A: C, 55.06; H, 4.69; N, 6.27; S, 7.18. Calcd. for $C_{21}H_{20}O_8N_2S$: C, 54.78; H, 4.38; N, 6.09; S, 6.97. Found for B: C, 58.20; H, 3.50; N, 4.55. Calcd. for $C_{16}H_{11}O_7N$: C, 58.36; H, 3.37; N, 4.26.

Alkaline Hydrolysis.—To 57 mg. of A in 10 cc. of dioxane was added 25 cc. of 0.02 *N* sodium hydroxide solution. After ten minutes, the solution was titrated to *p*H 8.0 with 0.02 *N* hydrochloric acid. The reaction involved utilization of 0.345 milliequivalent of alkali or one milliequivalent for 165 mg. of A. The solution now displayed a green fluorescence in ultraviolet light and had lost antibacterial activity.

Compound B was unchanged upon treatment with excess alkali; a solution of 15 mg. in 5 cc. ethanol-10 cc. water showed a neutralization equivalent of 337. The tentative formula has a molecular weight of 329.

TABLE II

APPROXIMATE CONCENTRATIONS OF CYSTEINE IN MG. PER CC. REQUIRED TO COMPLETELY INACTIVATE 0.06 MG. OF A PER CC. IN THE TIME PERIODS SHOWN AT THREE *p*H VALUES

*p*H	0.5 hr.	2 hr.	5 hr.	24 hr.
6	..	..	10	1
7	10	7	2	<0.5
8	4	2	[a]	[a]

[a] In this period of time, some loss in activity resulted in the controls from *p*H alone. No measurable losses resulting from *p*H were observed in the other tests.

(5) Analyses of this compound performed by Dr. Carl Tiedcke.

Inactivation by Cysteine.—Neutral solutions of cysteine readily inactivated B without change in color. The effect of *p*H, time of reaction and concentration of cysteine required to inactivate A are illustrated in Table II. The cylinder-plate method was used to determine activity against *S. aureus*.

Antibacterial Tests.—Compound A was dissolved in 50% ethanol in concentrations of 0.5 and 1.0 mg. per cc. These solutions were sterilized by filtration through an ultra-fine glass filter. The more dilute solution was used in testing antibacterial action against the aerobes while the other was used for the Clostridia. Decreasing amounts of the sterile solution containing 0.5 mg. per cc. (or decimal dilutions thereof) were added to series of tubes containing appropriate amounts of sterile culture medium so that the final volume in each tube was 4 cc. To these was then added 1 cc. of inoculum consisting of a 1:1000 dilution in medium of an 18-hour broth culture of the test organism. The aerobic bacteria and *Strep. fecalis* were tested in nutrient broth while the pneumococci and other streptococci were tested in a veal infusion broth containing 10% horse serum and 0.1% glucose. This latter medium appears to give lower values for antibacterial action with A, probably as a result of the high protein content's removing some of A by adsorption and preventing it from exerting its antibacterial action.

In testing the anaerobic bacteria, Bacto-Anaerobe Medium with Dextrose was the medium used. The appropriate amounts of compound A were placed in series of tubes and to these were added such volumes of hot (50°) anaerobe medium as to give a volume in each tube of 9 cc. The tubes were inoculated by adding to the lower portion of the medium 1 cc. of a 1:1000 dilution (in anaerobe medium) of an eighteen-hour culture of the organism. In this manner, mixing of the medium and A was effected under anaerobic conditions.

Compound B was tested by the same methods. A concentration of 0.1 mg. per cc. completely inhibited growth of *B. subtilis* S 8, *B. cereus* and *Staph. albus* 151 and partially inhibited *Strep. fecalis* and *Staph. aureus* 209; 0.075 mg. per cc. allowed normal growth of the latter pair and partial growth of the former group.

Discussion

The reactivity of A and B toward cysteine serves as a further illustration of antibiotics belonging to a class which may act by reacting with —SH groups essential for bacterial growth. The greater antibacterial activity of A than B might result from a greater tendency of A to be adsorbed by the bacterial enzymes (thus concentrated in the vicinity of —SH groups) or by involvement of ester or lactone groups (absent in B) with amino groups in the vicinity of the reacting —SH groups. A discussion of such possible reactions with lactones has been described elsewhere.[6]

Investigations will be continued as more material becomes available.

Acknowledgment.—We wish to thank Mr. William F. Warner for the cylinder-plate tests mentioned in this paper.

Summary

Two new antibiotic agents, A and B, have been isolated from the Wild Ginger, *Asarum canadense* var. *reflexum*. These have been assigned tentative empirical formulas of $C_{21}H_{20}O_8N_2S$ for A and $C_{16}H_{11}O_7N$ for B. Both substances are active against Gram positive bacteria and A is much

(6) Cavallito and Haskell, THIS JOURNAL, **67**, 1991 (1945).

more active than B. The substances are readily inactivated by cysteine and belong to the thiol-reactive group of antibiotics.

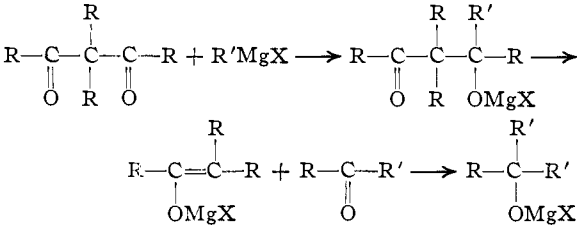
RENSSELAER, NEW YORK RECEIVED JANUARY 3, 1946

[CONTRIBUTION FROM THE COATES CHEMICAL LABORATORY OF LOUISIANA STATE UNIVERSITY]

The Cleavage of Cyclic Beta Diketones. I. Tetramethyl-1,3-cyclobutanedione[1]

BY J. L. E. ERICKSON AND GARRY C. KITCHENS[2]

It has been shown that various types of β-diketones[3] undergo a characteristic cleavage when treated with organic magnesium compounds



The ketone, formed as a primary cleavage product, does not survive in the presence of excess reagent and is converted readily to a tertiary alcohol.

Cyclic β-diketones have been reported to differ from open-chain β-diketones in their behavior toward the Grignard reagent.

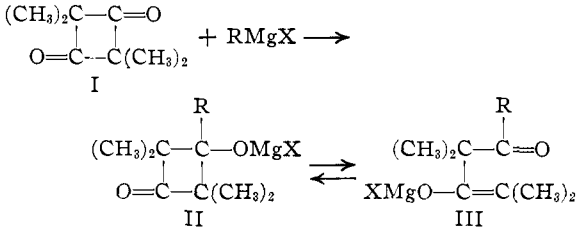
Wedekind and Miller[4] added tetramethyl-1,3-cyclobutanedione to an excess of ethylmagnesium bromide and obtained a compound, b. p. 128–130° (30 mm.), presumably the corresponding glycol resulting from the normal addition of two moles of the reagent.

Hurd, Jones and Blunck[5] reported that ethyl 1,3 - diethyl - 2,4 - cyclobutanedione - 1,3 - dicarboxylate probably adds two moles of Grignard reagent to form a diaddition product, which subsequently undergoes hydrolytic rupture of the ring to yield a β-keto ester.

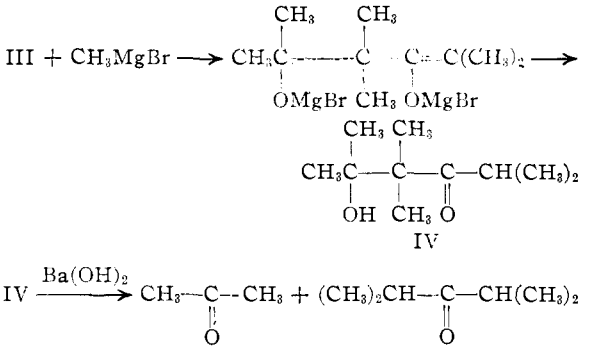
Geissman and co-workers[6] have observed that 2,2-dimethyl-1,3-indanedione and 8,8-dimethyl-7,9-perinaphthindanedione, compounds in which the two carbonyl groups are members of five and six-membered rings, respectively, undergo normal addition when treated with phenylmagnesium bromide.

Contrary to the report of Wedekind and Miller, experiments in this Laboratory with tetramethyl-1,3-cyclobutanedione have shown conclusively that the reaction of this compound with a variety of organic magnesium and lithium compounds (*e. g.*, methyl, ethyl, phenyl and mesitylmagnesium bromide, phenyllithium and mesityllithium) leads to cleavage of the ring in every case where any reaction at all took place.

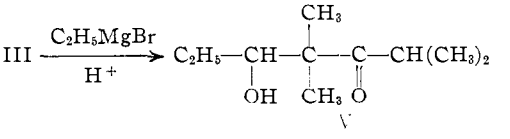
Tetramethyl-1,3-cyclobutanedione (I) reacted with an excess of Grignard reagent to yield a monoaddition product (II), which was unstable and underwent a reverse aldol condensation, resulting in cleavage of the ring with the formation of a primary cleavage product (III).



In the presence of an excess of methylmagnesium bromide, III did not survive and was converted into the β-hydroxy ketone, 5-hydroxy-2,4,4,5-tetramethyl-3-hexanone (IV), together with small quantities of acetone and diisopropyl ketone. When treated with barium hydroxide, IV decomposed almost quantitatively into acetone and diisopropyl ketone.



In the same manner it was found that ethylmagnesium bromide produced cleavage of I, yielding the primary cleavage product (III), which subsequently was reduced by the reagent to form 5-hydroxy-2,4,4-trimethyl-3-heptanone (V). The



(1) Partly from a thesis submitted by Garry C. Kitchens to the Faculty of Louisiana State University, June, 1945, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

(2) Present address: Givaudan-Delawanna, Inc., Delawanna, N. J.

(3) Kohler and Erickson, THIS JOURNAL, **53**, 2301 (1931).

(4) Wedekind and Miller, *Ber.*, **44**, 3285 (1911).

(5) Hurd, Jones and Blunck, THIS JOURNAL, **57**, 2033 (1935).

(6) (a) Geissman and Tulagin, *ibid.*, **63**, 3352 (1941); (b) Geissman and Morris, *ibid.*, **66**, 716 (1944).