mixtures were noted. Mixtures of benzoic, cinnamic, and citric acids showed a potentizing effect of from 34.4 to 53.7% in various percentage mixtures. This study verified the report of Schelhorn (5) that in the presence of citric acid less benzoic acid was required to inhibit the growth of microorganisms than when benzoic acid was used alone. The Petri dish tests indicated that benzoic acid alone would inhibit growth at 1:1030 while an equal mixture of benzoic and citric acids would inhibit at 1:830, or a potentizing effect of 61.1%.

Storage Tests Using Mixtures of Preservatives.—It was found that a mixture of butylparaben 50% and methylparaben 50% at 1:4500 would be best for preservation of Simple Syrup, U. S. P. At this concentration the mixture inhibited growth of molds in both the direct sun rays and the dark storage. At current prices the cost of the parabens for preserving one gallon of Simple Syrup, U. S. P. would be approximately one cent.

In the diluted simple syrup (42.5% sucrose w/v) several mixtures were found to inhibit the growth of molds, but only two could be used in low concentrations. They are butylparaben 90% and propylparaben 10% at 1:8000, and butylparaben 65% and propylparaben 35% at 1:7500. The cost of the parabens for preserving one gallon of diluted simple syrup (42.5% sucrose w/v) would be less than one cent.

SUMMARY

Storage tests indicated that the concentration of the preservatives which had been found to prevent growth in Petri dish tests was not sufficient to prevent growth of molds in four different storage conditions in Simple Syrup, U. S. P. or diluted simple syrup (42.5% sucrose w/v).

Tests showed that there was a definite potentizing action among the parabens, but that the increase was not very great. There was only a slight potentizing action between benzoic and cinnamic acids, but when citric acid was added there was a large potentizing effect.

Storage tests using mixtures of preservatives showed that for Simple Syrup, U. S. P. a mixture of 50% butylparaben and 50% methylparaben at 1:4500 was the best for the inhibition of molds. In diluted simple syrup (42.5% sucrose w/v) the use of either butylparaben 65% and propylparaben 35% at 1:7500, or butylparaben 90% and propylparaben 10% at 1:8000 was effective.

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The Isolation of Three Inositols from *Vernonia Altissima**

By EDWARD J. ROWE, ARTHUR A. HARWOOD, and DONALD B. MYERS

The isolation of *levo*-inositol, *meso*-inositol, and scyllitol from an aqueous extract of the air-dried leaves of *Vernonia altissima* is reported. *Meso*-inositol was isolated from a basic lead acetate precipitate, and *levo*-inositol and scyllitol from the deionized filtrate by fractional crystallization.

Vernonia altissima, Nutt. is one of more than 500 species of *Vernonia* widely distributed in warm-temperature regions (1). It is one of at least eight species (1) growing in the United States, blossoming from July through September. The plant is a composite and is commonly known as "Tall Iron-weed."

The leaves, roots, and seeds of some species of *Vernonia* have been ascribed medicinal properties and some have yielded physiologically active constituents (2–5). However, a literature survey revealed no particular therapeutic application of the *altissima* species nor any chemical study relative to isolation of constituents. An investigation of this plant has been, therefore, undertaken. In this report we present the isolation of three isomeric cyclitols, *meso*-inositol, scyllitol, and *levo*-inositol.

In the course of preliminary selective solvent extractions of the air-dried leaves, it was observed that the alcoholic extract after standing for several months contained a few clusters of well-defined crystals. The crystals were readily soluble in water. Therefore a water extract was prepared and subjected to a general lead process. Based on the weight of the air-dried leaves, yields of 0.08 per cent of *meso*-inositol, 0.2 per cent of scyllitol and 0.4 per cent of *levo*-inositol were obtained.

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EXPERIMENTAL

The leaves of *Vernonia altissima*, Nutt. were collected at the blossoming stage in the environs of Indianapolis, Indiana, and authenticated by the late Dr. Ray C. Friesner of the Botany Department, Butler University. The leaves were air dried and ground in a Wiley Drug Mill through a screen with 2-mm. perforations.

Water Extraction.—Five hundred grams of the ground leaves was placed in a wide-mouthed jar, covered with 3 L. of warm water, and mechanically shaken for three hours. The contents was then transferred to a percolator. Three liters of a chocolate-colored percolate was collected by passing an additional 1500 cc. of warm water through the marc. The percolate was immediately filtered through a pad of pulped filter paper on a Büchner funnel.

Lead Precipitation.—The percolate was treated at room temperature with 400 cc. of a saturated solution of lead acetate. The dense yellow precipitate formed was filtered off, washed, and set aside. The clear yellow filtrate together with the washings was concentrated at 55–60° to 1 L., filtered, and treated with 400 cc. of a 25% solution of lead subacetate. A dense bright yellow precipitate formed. The whole was boiled gently for ten minutes and allowed to cool. The lead subacetate precipitate (A) was filtered off and washed with several portions of hot water. The filtrate (B) therefrom together with the washings totaled 2 L.

Isolation of meso-Inositol.—The lead subacetate precipitate (A) was suspended in 700 cc. of water and treated with hydrogen sulfide. The lead sulfide was removed by filtration and washed with three 200-cc. portions of hot water. The filtrate together with the washings from the lead sulfide was deionized with Amberlites IR-120(H) and IRA-400(OH), and the nearly colorless effluent evaporated at 55–60° to dryness. The tan-colored residue remaining was washed with three 20-cc. portions of cold absolute methanol which removed some of the coloring matter. The washed residue was then taken up with 25 cc. of water, treated with Norit®, and concentrated to 10 cc. Addition of three volumes of ethanol and one-tenth the total volume of ether yielded upon refrigeration colorless, glistening isometric platelets. The yield was 0.08% The product when recrystallized twice from 70% ethanol melted at 224–225°.[1] There was no depression of the melting point when mixed with authentic *meso*-inositol. A Scherer test for inositols (6) was positive.

Anal.[2]—Calcd. for $C_6H_{12}O_6$: C, 40.00; H, 6.70. Found: C, 40.32; H, 6.94.

The hexaacetate was prepared in the usual manner by the use of acetic anhydride and anhydrous sodium acetate. The crude derivative was purified by several crystallizations from 70% ethanol. The crystals were colorless, pleochromic prisms, somewhat resembling mica. They melted at 215–216° which is in agreement with that recorded for *meso*-inositol hexaacetate.

Anal.—Calcd. for $C_{18}H_{24}O_{12}$: C, 50.00; H, 5.60. Found: C, 50.16; H, 5.58.

Isolation of Scyllitol.—The 2 L. of filtrate (B) and washings from the precipitation of lead subacetate was deionized with Amberlites IR-120(H) and IRA-400(OH). The practically colorless effluent was concentrated at 55–60° to 30 cc. at which volume it was amber in color. An equal volume of boiling ethanol was then added. Granular crystals of scyllitol began to separate out immediately. Separation was considered complete at the end of two days. The crystals were removed from the mother liquor (C). The yield was 1.10 Gm. When crystallized from hot water, glistening, colorless, rhombohedral prisms were obtained which melted at 346–347°[3] with decomposition. Reported melting points for scyllitol 345° (7), 348.5° (8), 352° (9), 353–355° (10).

Anal.—Calcd. for $C_6H_{12}O_6$: C, 40.00; H, 6.70. Found: C, 39.87; H, 6.81.

The hexaacetate was prepared by refluxing the crystalline scyllitol for fifteen hours with acetic anhydride and anhydrous sodium acetate. The crude product when crystallized several times from glacial acetic acid and washed thoroughly with water yielded colorless, glistening, pleochromic prismatic crystals melting at 291–291.5°. Reported melting points for scyllitol hexaacetate: 290–291° (8), 291° (11, 12), 296–297° (7), 299–300° (10), 300° (9).

Anal.—Calcd. for $C_{18}H_{24}O_{12}$: C, 50.00; H, 5.60. Found: C, 49.25; H, 5.54.

Isolation of levo-Inositol.—To the mother liquor (C) which yielded the scyllitol was added one and one-half its volume of hot ethanol. Slow crystallization of *levo*-inositol began after several hours and was considered complete in a week. The mother liquor was decanted and the clusters of crystalline material washed with ethanol and taken up in boiling 70% ethanol. The hot solution was then decanted from a slight amount of insoluble material, refluxed with Norit®, filtered, and allowed to stand for two days at room temperature and then for several more in a refrigerator for crystallization. Seventy-five per cent ethanol appeared to be the most satisfactory solvent for recrystallization. Two grams of crystals were obtained. From ethanol, the crystals were colorless, tetra- and octahedral prisms. The optical rotation was *levo*; $[\alpha]_D^{25} = -65°$ (water). Reported for *levo*-inositol; −65° (13, 14), −64.1° (15, 16), −63.8° (7). The melting point was 246–247°. Reported for *levo*-inositol: 245–250° (16), 247° (15), 249–250° (7). A Scherer test for inositols (6) was positive.

Anal.—Calcd. for $C_6H_{12}O_6$: C, 40.00; H, 6.70. Found: C, 40.68; H, 6.85.

The hexabenzoate was prepared from the crystalline *levo*-inositol using benzoyl chloride and pyridine (16). Recrystallization of the crude product several times from absolute ethanol yielded colorless, pleochromic, prismatic needles melting at 251–252°. Reported for *levo*-inositol hexabenzoate; 252° (13), 253–253.5° (16). The optical rotation was $[\alpha]_D^{25} = -67.7°$ (chloroform). Reported for *levo*-inositol hexabenzoate: −67.7° (16).

Anal.—Calcd. for $C_{48}H_{36}O_{12}$: C, 71.63; H, 4.51. Found: C, 70.94; H, 4.15.

[1] The melting points were determined with a Fisher-Johns apparatus and are uncorrected.

[2] The authors are indebted to Mr. W. L. Brown, Department of Organic Chemistry, Eli Lilly and Company, Indianapolis, Ind., for all of the combustion analyses.

[3] The melting points for scyllitol and scyllitol hexaacetate were determined in capillaries with an Anshütz thermometer.

SUMMARY

1. *Meso*-inositol, scyllitol, and *levo*-inositol have been isolated from an aqueous extract of the leaves of the "Tall Iron-weed."

2. *Meso*-inositol was obtained from the lead subacetate precipitate; scyllitol and *levo*-inositol by fractional crystallization of the deionized and concentrated filtrate therefrom.

3. By weight of air-dried leaves, yields of 0.08 per cent *meso*-inositol, 0.2 per cent scyllitol, and 0.4 per cent *levo*-inositol were obtained.

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The Synthesis and Investigation of Some Ethylene bis-Dithiocarbamate Esters as Fungicides*

By ALFRED P. COLLINS and GAIL A. WIESE

Some esters of ethylene bis-dithiocarbamic acid were synthesized by condensation of disodium ethylene bis-dithiocarbamate with the desired alkyl halide. These compounds were screened for antimycotic activity against five fungi pathogenic to man. A comparison of their activity to that of undecylenic acid was made. The diallyl ester was superior to undecylenic acid in the case of each organism. The bis-2-hydroxyethyl, bis-3-hydroxypropyl and the di-*n*-propyl esters approximated the action of undecylenic acid against several of the organisms.

SULFUR and its inorganic compounds have been recognized for many years as important and useful chemotherapeutic agents for the treatment of fungus diseases of plants, however, in more recent years workers in the field of plant pathology have focused their attention on the study of organic derivatives of sulfur, the first of which were introduced around 1930. Some of the most promising of the numerous organic sulfur compounds that have been studied by plant pathologists are the derivatives of dithiocarbamic acid, i. e., its salts, and the thiuram mono- and disulfides. Because of the intensive investigation that has been given these derivatives as agents for control of plant disease, particularly in view of the broad antimicrobial spectrum they have demonstrated, it is surprising the relatively slight attention they have received from investigators in the field of human chemotherapy.

Investigations since 1931 have shown some derivatives of dithiocarbamic acid to have insecticidal, fungicidal, and disinfecting properties (1–3). The activity of several of these compounds against human pathogenic fungi was first noted in the research laboratories of Bauer and Black in 1938 by Hall. Beginning in 1943 studies on dithiocarbamic acid and other thio-derivatives by Miller and Elson (4) revealed that with each type of derivative, dithiocarbamate, thiuram monosulfide, and thiuram disulfide, the lower members, i. e., the N-methyl and N-ethyl compounds, are the most active and the higher alkyl derivatives are relatively inactive. Kligman and Rosenweig (5) have found on an *in vitro* comparison that the dithiocarbamates possess lower LD_{50} and much greater activity in the presence of blood than do the naphthoquinones, some of which are at present used for the treatment of human fungus disease. Patch tests on rabbits and human subjects were also reported and from the data accumulated the most promising compounds were found to be among the dithiocarbamates. Unpublished findings reviewed by Stedman (6) confirmed in part the data of Kligman and Rosenweig regarding *in vitro* activity of some of the dithiocarbamates but revealed serious disadvantages to pharmaceutical adaptability. Although Kligman and

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