The Availability of Nordihydroguaiaretic Acid Antioxidant*

ALADAR FONYO
Wm. J. Stange Company, Chicago, Ill.

IN the past three years a great deal of time and energy have been devoted to the study of the antioxidating properties of Nordihydroguaiaretic Acid. Several articles appeared in OIL & SOAP dealing with this antioxidant as applied to fats and oils. Innumerable tests were conducted by food manufacturers all over the country with the application of Nordihydroguaiaretic Acid to their own products. Many of them reported very encouraging results and stated that their interest in this antioxidant was not merely academic but wanted to know if it were available when they were ready to use it. Since the availability of a product depends on the supply of raw materials and on the facilities for producing it, the discussion of the source of Nordihydroguaiaretic Acid and the methods of its recovery and purification is timely and of general interest.

The Natural Occurrence of Nordihydroguaiaretic Acid

The plant in which Nordihydroguaiaretic Acid was discovered in 1942 grows abundantly right in our own country. Millions and millions of acres of arid land in Texas, New Mexico, Arizona, California, Nevada, Colorado, and in Old Mexico are literally covered with this plant. It is commonly called the Creosote Bush because the leaves and twigs are covered with a resinous substance emitting an odor resembling creosote. The Mexicans call it hideondilla which translated means "the little bad smeller." Other popular names given by the natives are el gobernadora, palo ondo, sonora hideonodo, etc. I did not ask for a translation of these names for fear they might be more descriptive than hideondilla.

The plant is the only one known to contain Nordihydroguaiaretic Acid. It was first described by Cavanilles in 1800, who named it Larrea divaricata. De Condolle and other botanists also described the same plant, giving it various names such as Zygophyllum Tridentatum, Larrea Mexicana, Zygophyllum Californicum, Larrea Glutinosa, etc. The confusion of names was eventually clarified by Coville in 1893, who proved that all these plants were identical and suggested the name of Larrea Tridentata. Botanist Vail again changed it to Covillea Tridentata to honor Coville. The International Code of Botanical Nomenclature recognizes the name first published, thus Larrea divaricata is now official.

The creosote bush is an evergreen shrub. Its leaves are dark green, its stems dark brown, almost black. The shrub reaches a height of five to six feet and in some wind protected areas even a height of 10 to 12 feet. In April and May the bush bursts into full flower, which soon thereafter develops into fluffy white seed balls. During late summer the leaves acquire a brownish hue. The fruits drop, and the plant remains in a condition known as drought dormancy. It grows well in arid soil without irrigation in many districts where no other desert plant can exist. Therefore, it is a desirable shrub from the standpoint of ecology as it helps prevent soil erosion.

Indian Medicine Leads to Discovery of Nordihydroguaiaretic Acid

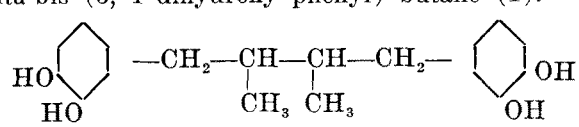
The Indians used the plant as a cure for a great many ailments. When the leaves were boiled in water, the decoction so produced was taken internally for venereal diseases, colds, intestinal cramps, gastric disturbances, inflamation of the respiratory tract, tuberculosis, etc. It also served as a tonic, as an antiseptic, and a stimulating expectorant. When the leaves were soaked in water, the liquid was used as a bath to cure rheumatism, chicken pox, etc. Another form of application was in drying and pulverizing the leaves and dusting the powder on wounds and abrasions. Occasionally, the condensed decoction was mixed with Badger Oil and used as salve for burns.

The literature does not disclose any pharmacological investigation of the plant. Its efficacy or lack of it, in the practice of Indian medicine, is now immaterial, but the fact that it had been used for that purpose is very fortunate as it made the plant eligible to be included in the Indian Medicinal Plant Study instituted by the Bureau of Plant Industry, United States Department of Agriculture, in cooperation with the University of Minnesota under the direction of Professor Raymond Bieter. The phytochemical study of the plant was under the direction of Professor Ole Gisvold of the College of Pharmacy and assigned to Coy H. Waller in preparation of his thesis. The result of this study was the isolation and identification of Nordihydroguaiaretic Acid as an ingredient of the Creosote Bush.

While these studies were progressing at the College of Pharmacy, the Hormel Research Foundation of the University of Minnesota was investigating other plants for natural antioxidants. The newly identified Nordihydroguaiaretic Acid was recognized by Professor H. O. Halvorson, Director of the Hormel Institute, and Dr. Lauer as a potential powerful antioxidant.

Nordihydroguaiaretic Acid

The Molecular Formula is $C_{18}H_{22}O_4$. Its structural formula is described as beta gamma dimethyl, alpha delta-bis (3, 4 dihydroxy phenyl) butane (1).



For convenience, the compound will hereafter be referred to by its trade-name N.D.G.A.

In a purified form N.D.G.A. is a white crystalline powder of a yellowish hue. Under the microscope it appears as flat needles. It has a melting point of 184°C. It is very soluble in alcohol and ether, only slightly soluble in chloroform, in hot benzine, toluene,

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or in hot water. It is insoluble in cold water or dilute hydrochloric acid.

N.D.G.A. is readily soluble in fats and oils at 120°C. As much as 5% may be dissolved at this temperature, but only about 1% remains in solution when allowed to cool and kept at room temperature.

Other Extractives of Larrea Divaricata

Maceration of the dried disintegrated plant with alcohol, ether, or other organic solvents yields a high percentage of extracted substances. Some of them were isolated and identified along with N.D.G.A. It contains a wax, an ester of C_{26} and C_{28} acids and of a C_{30} alcohol. It contains a yellow and orange coloring substance both of which were classified as flavonols. It contains caoutchouc (crude rubber), a volatile oil, phytosterol, sucrose, chlorophyll, and numerous phenolic compounds. A comparatively small portion of these is N.D.G.A.

Gisvold Process of Extraction

It was apparent that the usual process of extraction with organic solvents did not offer a practical method for producing N.D.G.A. The first problem was to obtain an extract of the plant material that contained a high percentage of N.D.G.A. and less of the undesirable ingredients. Gisvold directed his attention towards the use of a dilute aqueous solution of an alkali. Caustic soda forms with the acidic phenols very readily soluble salts and does not leach out much of the highly troublesome substances of the plant material, but it is very destructive to N.D.G.A. It was essential that the degradation of N.D.G.A. during the extraction process be averted. Gisvold added a powerful reducing agent, sodium hydro sulphite, to the alkali solution and succeeded in preventing substantially the decomposition of N.D.G.A.

The leaves and twigs of the Creosote Bush are collected, dried, and disintegrated. They are macerated in large vessels arranged in a series with a solution of about 5% of sodium hydroxide and 2 to 3% sodium hydro sulphite. Applying the counter-current principle to the extraction, this solution is pumped successively into the other vessels of the series. The alkali solution from the last tank is then transferred into a suitable container where it is immediately acidulated with a concentrated hydrochloric acid solution. A heavy precipitate forms which rapidly settles. The acid precipitate is a highly viscous substance called N.D.G.A. sludge. It contains from 10-15% N.D.G.A.

The sludge is then dissolved in methyl alcohol or in some other low chain water soluble aliphatic alcohol. From this alcoholic solution N.D.G.A. is transferred into diethyl ether or isopropyl ether. This ether solution then contains N.D.G.A. as well as other acidic substances. By fractional extraction with Gisvold's sodium hydroxide and sodium hydrosulfite solution N.D.G.A. is separated in the following manner: The calculated amount of Gisvold's solution is divided into as many as 22 portions. Each portion is added successively to the ether extract and agitated for several minutes, then allowed to settle. The aqueous layer is removed and acidulated with hydrochloric acid, and the precipitate so formed is removed.

The first four fractions yield an oily substance of no value. The 5 to 7 fractions produce a reddish brown solid material with very little N.D.G.A. The 8 to 17 batches yield crystalline substances with a high percentage of N.D.G.A. content. The 18 to 21 fractions are often contaminated with a considerable amount of impurities. The last fraction contains an oily substance and no N.D.G.A. The middle fractions, usually 8 to 17 contain between 90-95% N.D.G.A. They are collected for further purification.

The application of this process for large scale production of N.D.G.A. presented considerable difficulties, therefore Dr. Gisvold continued his experiments to develop a simpler method of extraction. After two years of arduous tasks he succeeded in eliminating the cumbersome process of fractionation. This new process, combined with the method of purification developed by Joseph Adams of the Wm. J. Stange Company, is now in operation producing N.D.G.A. of a high purity, free from odoriferous and coloring substances. It is regretted that, being in the patent applied for stage, this new process cannot be discussed at this time.

Seasonal Variation of N.D.G.A. Content of Creosote Bush

While the development work with the production and purification of N.D.G.A. was conducted, the sludge production went on uninterruptedly, which not only provided us with a good backlog of raw material but with valuable information regarding the characteristics of the Creosote Bush itself. The sludge is produced in Texas in the desert area, in the midst of a rich growth of Creosote Bush. Continuous production was started in May, 1944. From then until October we obtained a yield of sludge which we considered normal. The yield from November until March dropped sharply indicating a possible seasonal variation in the N.D.G.A. content of the plant. To ascertain this, an arrangement was made with the University of New Mexico to investigate the N.D.G.A. content of the Creosote Bush in various seasons of the year. It may be necessary to provide manufacturing facilities for sufficient sludge production in the summer months to take care of the entire year's requirements.

Distribution of N.D.G.A. in the Creosote Bush

Most of the N.D.G.A. is deposited on the surface of the leaves and small twigs, along with resins, gums, and wax. There is no appreciable amount of N.D.G.A. on the surface of the large branches or within their cellular structure. Collection of raw material is then restricted to picking leaves or rather to cutting the small twigs with the leaves. The bush and the large branches remain intact. The operation corresponds to nothing more than pruning of the bush with the result that new shoots sprout with a more luxuriant growth of leaves than the old ones. It is a unique case of eating your cake and having more of it.

The cutting of the leaves and twigs of the creosote bush is a tedious chore as it is being done by hand. However, we are experimenting with various devices to do it mechanically. Now, the job is done mostly by Mexicans, but we are trying to secure the assistance of the Indians on various reservations but it takes high pressure salesmanship to persuade the men to let their squaws do the work.

Conclusion

The antioxidative efficiency of N. D. G. A. is extremely high; as reported by numerous investigators

0.002%-0.005% concentration with suitable synergists is sufficient to protect fats for all practical purposes (2). Therefore, the anticipated demand is not of great volume. Raw material and facilities for production are ample to take care of the demand.

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The Diffraction of X-Rays by Stearates of Calcium, Barium and Magnesium*

GEROULD H. SMITH and SYDNEY ROSS **
Department of Chemistry, Stanford University, California

THE commercial interest of calcium, barium, and magnesium soaps lies in their use in lubricating greases, as dusting powders, water-proofing agents, lubricants for rubber and plastic moulding, etc. The growing use of these compounds renders their analysis by x-ray diffraction of interest. The x-ray method also permits the investigation of reactions between the metallic stearates and the other constituents with which they may be mixed in commercial preparations. The present paper describes methods of preparation and of purification of some metallic stearates, reports the x-ray powder diffraction data by means of which qualitative analysis can be conducted, and investigates some reactions of commercial importance in the manufacture of lubricating greases.

Materials

The samples of magnesium chloride ($MgCl_2 \cdot 6H_2O$), calcium hydroxide [$Ca(OH)_2$], barium acetate monohydrate, and calcium acetate monohydrate which were used in this work were all Baker's C. P. Analysed Reagents. The barium hydroxide [$Ba(OH)_2 \cdot 8H_2O$] was Baker's Purified Reagent. Baker and Adamson Reagent Grade of calcium chloride ($CaCl_2 \cdot 2H_2O$) barium chloride ($BaCl_2 \cdot 2H_2O$) and glacial acetic acid were employed. By x-raying a sample taken directly from the reagent bottle, the calcium acetate monohydrate was found to be predominantly the anhydrous form mixed with the monohydrate.

Eastman Kodak stearic acid was used for the preparation of the samples by direct reaction. This acid had been recrystallized twice from acetone and had a neutralization value 197 mg. KOH/g. of acid (theory = 197.3).

The potassium stearate used for metathetical reactions with the metal chlorides was prepared in methyl alcohol. Equivalent amounts of alcoholic carbonate-free potassium hydroxide solution and Eastman Kodak stearic acid (neutralization value 198) were reacted and the soap formed was washed free of the alcohol, using acetone. The soap was dried to constant weight over phosphorus pentoxide.

Methods

Preparation of soaps by metathesis—The preparation of calcium stearate by metathesis between 0.7 g. of $CaCl_2 \cdot 2H_2O$ (a slight excess) and 3.0 g. of potassium stearate, each dissolved in 250 cc. of boiled-out distilled water, was carried out by slowly adding the potassium stearate solution to the calcium chloride solution at 60°C. During the addition of the soap solution the precipitation mixture was vigorously stirred, using a Medco Easy-Mix Blendor. The calcium soap was separated by filtration and washed with distilled water until the addition of silver nitrate solution to the filtrate produced no turbidity. The soap was then dispersed in acetone and filtered to extract free fatty acid and water, and washed with acetone until the filtrate was free from dissolved material. This soap was dried in air until light and powdery. Then it was dried to constant weight over phosphorus pentoxide in an evacuated desiccator.

Barium stearate was prepared by the same procedure using 1.2 g. of $BaCl_2 \cdot 2H_2O$ and 3.0 g. of potassium stearate, conducting the precipitation at 60°C.

A sample of magnesium stearate was prepared from 2.2 g. of $MgCl_2 \cdot 6H_2O$ and 3.0 g. of potassium stearate, in the manner described above, but the precipitation was conducted at 25°C. The lower precipitation temperature is necessary since aqueous magnesium chloride solution hydrolyzes on heating, yielding hydrochloric acid and eventually magnesium oxide.

Preparation of soaps by direct reaction—The direct reaction of 0.4 g. of calcium hydroxide with 4.0 g. of melted Eastman Kodak stearic acid (recrystallized from acetone) was used to prepare samples of calcium stearate. The mixture was heated until foaming had ceased, which indicated that most of the water of reaction had been driven from the batch. Then the soap was quenched under acetone and the hard soap mass ground while still in the acetone. It was filtered and the residue washed with fresh portions of acetone until the filtrate was free from dissolved material. The soap was dried in air and then dried to constant weight over phosphorus pentoxide.

Barium stearate samples were prepared in the same manner using barium hydrate and Eastman Kodak stearic acid.

Treatment of the metallic stearates with acetic acid—Portions of the calcium, barium, and magnesium stearates prepared by metathesis were each dispersed in acetone and an equivalent amount of glacial acetic acid added to each. They were warmed to about 40°C. while stirring and then filtered. The residue was washed with acetone until the filtrate contained no dissolved material. The salts were dried in air, powdered, and then redried to constant weight over phosphorus pentoxide.

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**Now associate professor of chemistry, University of Alabama, Montgomery, Ala.