Occurrence of Norcaperatic Acid in *Polyporus fibrillosus*

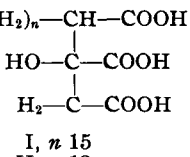
By GERALD SULLIVAN

Norcaperatic acid (α-tetradecylcitric acid) has been isolated from a carpophore of *Polyporus fibrillosus* Karst. in a 22 percent yield and identified by its physical and chemical properties. This is the first reported occurrence of norcaperatic acid in a *Polyporus* species.

THE NATURAL occurrence of aliphatic citric acid derivatives appears to be confined to a limited number of basidiomycetes and a lichen. Agaricic acid (α-hexadecylcitric acid) has been isolated from *Fomes officinales* (Fr.) Faull. [syn.: *Polyporus officinalis* Fr.] (1) and caperatic acid, the mono methyl ester of norcaperatic acid (α-tetradecylcitric acid), from the lichen *Parmelia caperata* L. (2). More recently Miyata *et al.* (3) found the potassium salt of norcaperatic acid to be present in the mushroom *Cantharellus floccosus* Schw. and suggested that this compound may be responsible for the reported toxicity of the mushroom.

Polyporus fibrillosus Karst. [syn.: *Pycnoporellus fibrillosus* (Karst.) Murr. and *Polyporus aurantiacus* Peck] is considered an uncommon mushroom and belongs to the order Polyporales (4). An examination of the literature for substituted citric acid derivatives occurring in the Polyporales revealed that only two such compounds, agaricic (1) and norcaperatic acid (3), had been reported. In addition to these two compounds, Birkinshaw *et al.* (5) reported the isolation of ungulinic acid, which bears a general structural relationship to these substituted citric acid derivatives, from the mycelium of *Polyporus resinosus* Fr. [syn.: *Polyporus benzoinus* (Wahl.) Fr.] which had been grown in saprophytic culture.

The structural formulas of agaricic acid (I) and norcaperatic acid (II) may be represented as:



I, n 15
II, n 13

EXPERIMENTAL[1]

Isolation and Characterization—A carpophore of *Polyporus fibrillosus* Karst. had been collected in the vicinity of Darrington, Wash., in September 1965. The carpophore was orange-red in color and was found attached to a fallen coniferous tree. The carpophore was dried in a forced-air drying oven at 48° for 48 hr., ground to a 60-mesh powder in a Wiley laboratory mill, and subjected to a selective solvent extraction in a Soxhlet apparatus employing a sequence of petroleum ether, ether, chloroform, and 95% ethanol.

A total yield of 31 g. (22%, dry weight basis) of a white microcrystalline material was obtained from the ether extract. The isolated material was found to be insoluble in cold water but soluble in 5% sodium hydroxide, boiling water, acetone, ethanol, and pyridine.

Partial purification of this material was accomplished by repeated recrystallization from boiling water, which resulted in a compound that exhibited a melting range of 132–135°. Final purification was obtained by solubilizing the compound in acetone, mixing with activated carbon, and filtering. The solvent was evaporated under pressure at 50° and the resulting compound recrystallized 3 times from boiling water. The compound was dried at room temperature and a portion of the isolated compound was further dried in a drying pistol for a 24-hr. period at 65°.

Qualitative elemental analysis revealed the absence of nitrogen, sulfur, and halogens and a m.p. of 137–138° was observed. A small amount of the compound was placed on a spatula and ignited in a flame. No residue was observed, indicating that the isolated compound was not a salt. An IR spectrum of the compound in a KBr pellet suggested the presence of (OH), (CH_2), and (C=O) groups: $\nu_{max.}$ 3,500, 2,850, and 1,720 $cm.^{-1}$. The modified Furth-Herrmann color reaction test (3, 6, 7) for aliphatic polycarboxylic acids was applied to the isolated compound and a positive test was obtained when a few milligrams of the compound was dissolved in 3 drops of pyridine and 3 ml. of acetic anhydride added.

The results of the IR spectrum, solubility, ignition, and color reaction tests of the isolated compound indicated that it was probably an aliphatic hydroxy carbonyl compound.

Identification of the Isolated Compound—Authentic samples of both agaricic and norcaperatic acid were obtained and a comparison of the isolated compound with those of the two authentic samples was conducted. The IR absorption spectrum of the isolated compound was found to be identical to that of norcaperatic acid. The observed m.p. (137–138°) of the isolated compound was identical to that of norcaperatic acid and a mixed m.p. with norcaperatic acid resulted with no depression of the original m.p. A parent peak was not obtained from the mass spectra of these compounds but the fragmentation

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[1] The melting points are uncorrected and were determined using a Thomas-Hoover Unit-Melt capillary melting point apparatus. The IR spectra were taken in KBr pellets using a Beckman infrared spectrophotometer, model IR5A. The NMR spectra were determined on a Varian A-60 spectrometer in pyridine with tetramethylsilane as internal standard. The mass spectra were obtained on a C.E.C. No. 21-103, and quantitative elemental analysis was performed by Schwarzkopf Microanalytical Laboratory.

patterns for both norcaperatic acid and the isolated compound were identical. Peaks at m/e = 29, 43, 57, 59, 85, 99, 113, 126, 299, 308, and 326 were consistent with the structure of the molecule. The NMR spectra of both the isolated compound and norcaperatic acid were found to be identical and were in agreement with that reported by Miyata *et al.* (3) for norcaperatic acid. A ratio of 3:29 was obtained by the integration of the combined signals of the protons on the α and γ carbons and the protons on the aliphatic side chain. The absence of a methyl ester function in the infrared, mass, and NMR spectra excluded the possibility of the isolated compound being caperatic acid.

The potassium salt of the isolated compound was prepared as described by Miyata *et al* (3). The observed m.p. (173–174°) of the prepared salt was identical to that reported for the potassium salt of norcaperatic acid. A mixed m.p. with a sample of the reference compound resulted with no depression of the original m.p.

Quantitative elemental analysis of the prepared salt gave the following results.

Anal.—Calcd. for $C_{20}H_{35}KO_7$: C, 56.31; H, 8.27. Found: C, 55.97; H, 8.33.

RESULTS AND DISCUSSION

A 22% yield of norcaperatic acid was obtained from the ether extract of *P. fibrillosus*. The identification of the acid was established by its solubilities, its physical and chemical properties, and by direct comparison with reference compounds. This marks the first reported occurrence of norcaperatic acid in a *Polyporus* species.

The very high concentration of norcaperatic acid in *P. fibrillosus* is reminiscent of the reported concentration of agaricic acid (18%, dry weight basis) in *F. officinalis*. There appears to be no apparent function for these compounds in the respective fungi other than that of waste products. Nord (8) has suggested that many of the metabolites produced in yields exceeding functional requirements, or for which there is no apparent function, accumulate because some of the enzyme systems involved in the oxidative sequence become saturated with respect to their substrates.

The occurrence of norcaperatic acid in the genus *Polyporus* may possibly be restricted to only *P. fibrillosus* since Overholts (4) indicated that this species has no close relatives and numerous other phytochemical investigations (9, 10) have not revealed the presence of this type of acid in other *Polyporus* species.

REFERENCES

(1) Thoms, H., and Vogelsang, J., *Ann. Chem.*, **357**, 145 (1907).
(2) Hesse, O., *J. Prakt. Chem.*, **57**, 409(1898)..
(3) Miyata, J. T., Tyler, V. E., Jr., and Brady, L. R., *Lloydia*, **29**, 43(1966).
(4) Overholts, L. O., "The Polyporaceae of the United States, Alaska, and Canada," University of Michigan Press, Ann Arbor, Mich., 1953, p. 382.
(5) Birkinshaw, J. H., Morgan, E. N., and Findlay, W. P. K., *Biochem. J.*, **50**, 509(1952).
(6) Casares-Lopez, R., *Biochem. Z.*, **284**, 365(1936).
(7) Roeder, G., *J. Am. Pharm. Assoc., Sci. Ed.*, **30**, 74 (1941).
(8) Nord, F. F., and Clarke, D. D., *Arch. Biochem. Biophys.*, **59**, 285(1955).
(9) Shibata, S., Natori, S., and Udagawa, S., "List of Fungal Products," University of Tokyo Press, Tokyo, 1964, p. 168.
(10) Miller, M. W., "The Pfizer Handbook of Microbial Metabolities," McGraw-Hill, New York, N. Y., 1961, p. 766.

Keyphrases

Norcaperatic acid—*Polyporus fibrillosus*
α-Tetradecylcitric acid—isolated, identified
IR spectrophotometry—identity
Mass spectroscopy—identity
NMR spectroscopy—identity

Utilization of the Guggenheim Method in Kinetics

By PAUL J. NIEBERGALL and EDWIN T. SUGITA

The Guggenheim method for the evaluation of rate constants is shown to be applicable to a wide range of problems that are of pharmaceutical interest. These include reaction kinetics in which more than one product is produced from a common reactant, consecutive first-order reactions, dissolution followed by partitioning into a lipid phase, the use of dissolution kinetics to obtain drug solubility, and the analysis of drugs through kinetics.

FIRST-ORDER chemical reactions are frequently followed by directly measuring x, the concentration reacted, and then obtaining the rate constant by plotting the logarithm of $a - x$ *versus* time, where a is equal to the initial concentration of the reactant. Alternately, some physical property, P, which is proportional to concentration may be used, and a plot of log $(P - P_\infty)$ or log $(P_\infty - P)$ *versus* time is used to obtain the rate constant. One objection to this is that overemphasis is placed upon the initial concentration or the time infinity reading of the physical property. A further difficulty arises when either the initial concentration or the final reading of the physical property cannot be obtained. In order

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