

Safety Data Sheet

Verrucarins

Division of Safety
National Institutes
of Health



WARNING!

COMPOUNDS IN THIS CLASS ARE HIGHLY TOXIC. THEY ARE READILY ABSORBED THROUGH THE INTESTINAL TRACT. THEY MAY CAUSE SEVERE IRRITATION OF TISSUES (SKIN, EYES, MUCOUS MEMBRANES, AND LUNGS) AND INDUCE SENSITIVITY. AVOID FORMATION AND BREATHING OF AEROSOLS.

LABORATORY OPERATIONS SHOULD BE CONDUCTED IN A FUME HOOD, GLOVE BOX, OR VENTILATED CABINET.

AVOID SKIN CONTACT: IF EXPOSED, WASH WITH SOAP AND COLD WATER. AVOID WASHING WITH SOLVENTS. AVOID RUBBING OF SKIN OR INCREASING ITS TEMPERATURE.

FOR EYE EXPOSURE, IRRIGATE IMMEDIATELY WITH LARGE AMOUNTS OF WATER. FOR INGESTION, INDUCE VOMITING. DRINK MILK OR WATER. REFER FOR GASTRIC LAVAGE. FOR INHALATION, REMOVE VICTIM PROMPTLY TO CLEAN AIR. ADMINISTER RESCUE BREATHING IF NECESSARY. REFER TO PHYSICIAN.

IN CASE OF LABORATORY SPILL, WEAR PROTECTIVE CLOTHING DURING CLEANUP. AVOID SKIN CONTACT OR BREATHING OF AEROSOLS. USE ETHANOL TO DISSOLVE COMPOUND. USE ABSORBENT PAPER TO MOP UP SPILL. WASH DOWN AREA WITH SOAP AND WATER. DISPOSE OF WASTE SOLUTIONS AND MATERIALS APPROPRIATELY.

A. Background

Verrucarins are metabolites of the fungus Myrothecium verrucaria (Albertini et Schweinitz) Ditmar ex Fries which grows parasitically on leaves of gardenia, tomatoes, violets, kidney beans, and other plants and on soil. They have been isolated from the culture broth and mycelium of this organism. Chemically they consist of three distinct classes of compounds:

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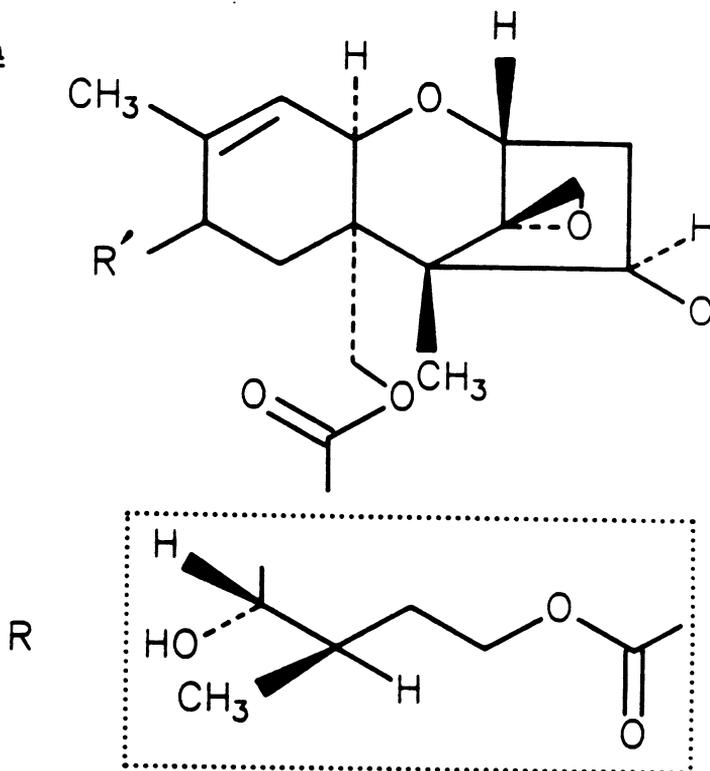
- a. verrucarins A, B, H, J, K, and L are macrocyclic triesters (tricothecenes) which are derivatives of the sesquiterpene alcohol verrucarol;
- b. verrucarins E, F, and G are nitrogen-containing compounds (E, and possibly F and G, are pyrrole derivatives); and
- c. miscellaneous compounds such as ergosterol and probably verrucarins C and D. Only the first class, which contains the major metabolites A and B, are included in this Data Sheet, and all references to "verrucarins" will denote this group only. These verrucarins are also closely related structurally to roridines, isolated from Myrothecium roridum Tode ex Fries and which are diesters of verrucarol.

Verrucarins are colorless crystalline compounds with no definite melting point but decompose above 250-300°C. They are antibiotic, larvicidal, antifungal, and cytostatic. In mammalian species they are toxic, highly irritating to skin and eyes, and on parenteral administration produce slow-healing ulcers.

Reviews of isolation and properties of some verrucarins (and roridines) include Härrri et al., 1962; Rüsck and Stähelin, 1965, Tamm, 1974.

B. Chemical and Physical Data

Structure of Verrucarin A



Verrucarin A^A

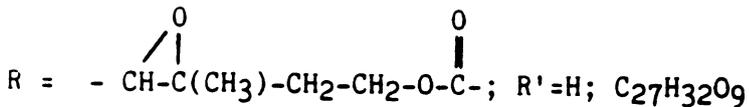
1. Chemical Abstract No.: 3148-09-2.

^AData which are common to all verrucarins are grouped together under "General properties," below.

2. Synonyms: Muconomycin A, NSC 126,728; Spiro (16, 18-methano-1H, 3H, 23H-(1,6,12)trioxacyclooctadecino (3,4-d)(1)benzopyran-17 (18H), 2'-oxirane)-3, 9, 14-trione, 4, 5, 6, 7, 16, 16a, 19a, 22-octahydro-4-hydroxy-5, 16a, 21-trimethyl-, stereoisomer. For complete stereoisomeric definition by name see Chem. Abstracts, 10th Collective Index, p. 55220CS.
3. Chemical structure and molecular formula: see above; R'=H; C₂₇H₃₄O₉
4. Optical rotation: $[\alpha]_D = +260^\circ$ in chloroform, $+208^\circ$ in dioxane.
5. Absorption spectroscopy: $\lambda_{\max} = 260$ nm (log $\epsilon = 4.25$); IR and UV spectra: see Härrri et al., 1962.
6. Melting point: Decomposes above 330°C.

Verrucarín B

1. Chemical Abstract No.: 2290-11-1
2. Synonym: 2'-deoxy-2'-3'-epoxyverrucarin A.
3. Chemical structure and molecular formula:



4. Optical rotation: $[\alpha]_D = +94^\circ$ in chloroform, $+101^\circ$ in dioxane.
5. Absorption spectroscopy: $\lambda_{\max} = 258.5$ nm (log $\epsilon = 4.37$). For UV, IR, and NMR spectra see Härrri et al., 1962 and Gutzwiller and Tamm, 1965.
6. Melting point: Decomposes above 330°C.

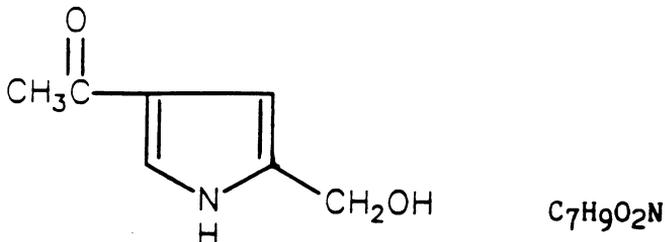
Verrucarín C: No information except mp = 223-4°C (may not belong to this class). IR spectrum: see Härrri et al., 1962.

Verrucarín D: No information except mp = 127-8°C (may not belong to this class).

Verrucarín E

1. Synonym: 3-acetyl-5-methoxypyrrole.

2. Chemical structure and molecular formula:



3. Absorption spectroscopy: λ_{max} ($\log \epsilon$) = 198 (4.02), 249 (4.0)
IR and NMR spectra: see Fetz and Tamm, 1966.

4. Melting Point: 103-104°C.

Verrucarin F: No information except mp = 237-238°C; $[\alpha]_{\text{D}} = -1$ (pyridine); $\lambda_{\text{max}} = 202, 233, 308$ nm. IR spectrum: see Härrri et al., 1962.

Verrucarin G: No information except $\lambda_{\text{max}} = 208, 254, 300$ nm. IR spectrum: see Härrri et al., 1962.

Verrucarin H: Chemical structure: unknown; molecular formula: $\text{C}_{29}\text{H}_{36}\text{O}_8$. No free hydroxyl groups. Decomposition temperature above 240°C. λ_{max} ($\log \epsilon$) = 195 (4.2), 223 (4.35), 259 (4.21). UV and IR spectra: see Böhner et al., 1965.

Verrucarin I:

1. Chemical Abstract No.: 71427-24-2

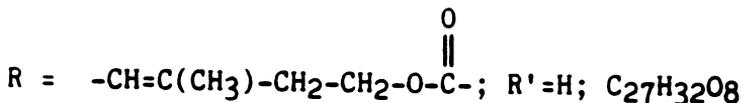
No other information.

Verrucarin J:

1. Chemical Abstract No.: 4643-58-7

2. Synonyms: Muconomycin B; 2',3'-dehydro-2'-deoxyverrucarin A.

3. Chemical structure and molecular formula:



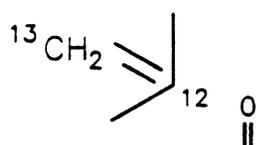
4. Absorption spectroscopy: NMR spectrum: see Fetz et al., 1965.

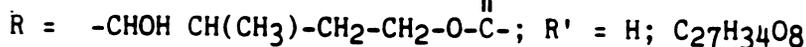
5. Melting point: Above 320°C; decomposition starts at 270°C (Böhner et al., 1965).

Verrucarin K

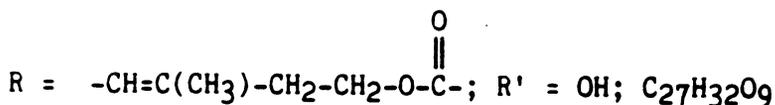
1. Chemical Abstract No.: No information.

2. Chemical structure and molecular formula:

Note: This is the only verrucarins so far characterized which does not contain the 12, 13-epoxide grouping shown in the basic structure. It is replaced by . (Breitenstein and Tamm, 1977).

Verrucarins L

1. Chemical Abstract No.: 77101-87-2
2. Synonyms: 2',3' didehydro-2'-deoxy-8-hydroxyverrucarin A; 8-hydroxyverrucarin J.
3. Chemical structure and molecular formula:



4. Absorption spectroscopy: $\lambda_{\text{max}} = 262 \text{ nm}$ ($\log \epsilon = 4.42$). For ^{13}C and ^1H NMR data see Jarvis et al., 1981.
5. Melting point: Decomposes above 230-235°C.

General properties of verrucarins

1. Chemical Abstract No.: 54018-05-2 (Class number).
2. Density: No data.
3. Volatility: No data but may be considered essentially nonvolatile.
4. Solubility: Very slightly soluble in water but may be brought into aqueous solution by addition of organic solvents. Soluble in ethanol, ether, chloroform, benzene, dioxane, and dimethyl sulfoxide.
5. Description: Colorless crystals.
6. Stability: No data but may be considered temperature-stable below 200°C.
7. Chemical reactivity: Base-catalyzed hydrolysis results in formation of verrucarol, cis, trans-muconic acid, and lactones characteristic of each verrucarins (see Tamm, 1974 for data on

verrucarin A, B, and J). Treatment with mineral acids causes cleavage of the oxirane (12,13-epoxy-) ring when present and rearrangement of the ring system.

8. Flash point: No data.
9. Autoignition temperature: No data.
10. Explosive limits in air: No data.

Fire, Explosion, and Reactivity Hazard Data

1. Because of the highly irritating properties of verrucarins, fire-fighting personnel should wear protective clothing and full face masks.
2. No incompatibilities are known.
3. Conditions contributing to instability are bases, acids, and high temperature.
4. Hazardous decomposition products include verrucarol (lower toxicity than verrucarins but similar irritating properties).
5. Nonspark equipment not required.

Operational Procedures

The NIH Guidelines for the Laboratory Use of Chemical Carcinogens describe operational practices to be followed when potentially carcinogenic chemicals are used in NIH laboratories. The NIH Guidelines should be consulted to identify the proper use conditions required and specific controls to be implemented during normal and complex operations or manipulations involving verrucarins.

It should be emphasized that this data sheet and the NIH Guidelines are intended as starting points for the implementation of good laboratory practices when using this compound. The practices and procedures described in the following sections pertain to the National Institutes of Health and may not be universally applicable to other institutions. Administrators and/or researchers at other institutions should modify the following items as needed to reflect their individual management system and current occupational and environmental regulations.

1. Chemical inactivation: No validated method reported.
2. Decontamination: Turn off equipment that could be affected by verrucarins or the materials used for cleanup. If there is any uncertainty regarding the procedures to be followed for decontamination, call the NIH Fire Department (dial 116) for assistance. Use absorbent paper to mop up spill. Wipe off surfaces with ethanol, then wash with copious quantities of

water. Glassware should be rinsed in a hood with ethanol, followed by soap and water. Animal cages should be washed with water.

3. Disposal: No waste streams containing verrucarins shall be disposed of in sinks or general refuse. Surplus verrucarins or chemical waste streams contaminated with verrucarins shall be handled as hazardous chemical waste and disposed of in accordance with the NIH chemical waste disposal system. Nonchemical waste (e.g., animal carcasses and bedding) containing verrucarins shall be handled and packaged for incineration in accordance with the NIH medical-pathological waste disposal system. Potentially infectious waste (e.g., tissue cultures) containing verrucarins shall be disinfected by heat using a standard autoclave treatment and packaged for incineration, as above. Burnable waste (e.g., absorbent bench top liners) minimally contaminated with verrucarins shall be handled as potentially infectious waste and packaged for incineration, as above. Absorbent materials (e.g., associated with spill cleanup) grossly contaminated shall be handled in accordance with the chemical waste disposal system. Radioactive waste containing verrucarins shall be handled in accordance with the NIH radioactive waste disposal system.
4. Storage: Store solid verrucarins and their solutions in dark-colored, tightly closed containers, preferably under refrigeration. Avoid exposure to light and moisture. Store working quantities of verrucarins and their solutions in an explosion-safe refrigerator in the work area.

Monitoring and Measurement Procedures Including Direct Field Measurements and Sampling for Subsequent Laboratory Analysis

1. Sampling: No data.
2. Analysis: There is no known procedure which can be applied to the analysis of verrucarins without a high degree of purification. Ultraviolet spectra are characteristic for the class but cannot be used to distinguish between their members and their metabolites. Thin-layer chromatography is useful in establishing the purity of components, and a list of R_f values in various solvents has been published (Tamm, 1974); however, TLC is very insensitive for analytical purposes. Two bioassay procedures specific for tricothecenes have been described which are based on incorporation of ¹⁴C-leucine in rabbit reticulocytes (Ueno and Shimada, 1974) and on cytotoxicity to two human epithelial type cells (Robb and Norval, 1983), respectively. Sensitivity of the latter method to verrucarin A is stated to be 0.1 ng.

Biological Effects (Animal and Man)

(Note: With very few exceptions all biological data have been obtained with verrucarins A; it is probable that these findings should pertain also, at least qualitatively, to other verrucarins of the tricothecene type.)

1. Absorption: Verrucarins produce toxic effects on ingestion and intracutaneous and parenteral injection. They produce intensely irritating effects when applied to skin and eyes but it is not known whether systemic effects are produced via these routes.
2. Distribution: No data.
3. Metabolism and excretion: No data.
4. Toxic effects: The acute LD50s of verrucarins A by intravenous administration in the mouse, rat, and rabbit are 1.5, 0.87, and 0.54 mg/kg, respectively (Rüsch and Stähelin, 1965); the intraperitoneal LD50 in the mouse of verrucarins A and J is in the range of 0.5-0.75 mg/kg (Guarino et al., 1968). A lamb given 4 mg/kg of verrucarins A by stomach tube died in 7 hours, while another at a dose of 2 mg/kg survived but showed severe mucosal erosion of the intestine (Mortimer et al., 1971). The toxic effects are slow in onset: mice injected with 33 mg/kg survived for several hours.

Chronic effects on prolonged intravenous administration of tolerated doses (0.08-0.15 mg/kg) to dogs, pigs, and monkeys produced leukocytosis followed by leukopenia and often thrombopenia. These effects were reversible on discontinuance of administration (Rüsch and Stähelin, 1965) without histopathology. Oral administration to dogs resulted in strong irritation of the gastrointestinal tract, while repeated intraperitoneal injection in rats produced peritonitis. Creatinuria has been noted in mice (Guarino et al., 1968).

Human exposure (e.g., laboratory workers involved in toxic culture extraction or animal handling) often results in dermatitis, usually of the face and especially of the soft skin of eyelids and orbits, with irritation, edema, and desquamation (Mortimer et al., 1971).

The mechanism of toxic action is not too well understood but appears to be mainly inhibition of protein synthesis initiation (Weil and McLaughlin, 1974; Liao et al., 1976). The demonstrated in vitro inhibition of creatine phosphokinase (Guarino et al., 1968) reflects the finding of creatinuria mentioned above.

In lower forms, the larvicidal effect against Aedes aegypti of verrucarins A and B was stronger than that of other tricothenes among 17 compounds tested (Grove and Hosken, 1975).

5. Carcinogenic effects: There is no evidence of any carcinogenic action of verrucarins. On the contrary, verrucarin A is strongly cytostatic in vivo against Ehrlich ascites, sarcoma 37, and others (Rüsch and Stähelin, 1965) and mouse B16 melanoma cells (Brinkerhoff and Lubin, 1977).
6. Mutagenic and teratogenic effects: No such effects have been described.

Emergency Treatment and Medical Surveillance

1. Skin and eye exposure: For skin exposure, remove contaminated clothing and wash skin with soap and water. Skin should not be rinsed with organic solvents. Avoid rubbing of skin or increasing its temperature. For eye exposure, irrigate immediately with copious quantities of running water for at least 15 minutes. Obtain ophthalmological evaluation.
2. Ingestion: Drink plenty of water or milk. Induce vomiting. Refer for gastric lavage.
3. Inhalation: Remove victim promptly to clean air. Administer rescue breathing if necessary.
4. Refer to physician at once. Consider treatment for pulmonary irritation.

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