Tetrahedron, Vol. 37, Supplement No. 1, pp. 379 to 385, 1981
Printed in Great Britain

0040-4020/81/Suppl. 1/0379-07 $02.00/0
Pergamon Press Ltd

THE STERPURIC ACIDS

A NEW TYPE OF SESQUITERPENOID†

WILLIAM A. AYER* and M. HOSSEIN SAEEDI-GHOMI
Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2G2

and

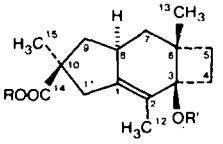
DONNA VAN ENGEN, BRUCE TAGLE and JON CLARDY*
Department of Chemistry, Baker Laboratory, Cornell University, Ithaca, NY 14853, U.S.A.

Abstract— The fungus *Stereum purpureum*, the cause of the so-called silver leaf disease, produces a new type of sesquiterpenoid when grown in liquid culture. The structures and reactions of sterpuric acid (**1**), methyl hydroxysterpurate (**17**), and methyl hydroxysterpurate ethylidene acetal (**22**) are reported. An X-ray crystallographic structure determination of sterpuric acid (**1**) is presented.

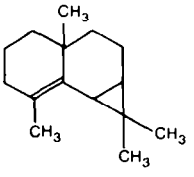
The fungus *Stereum purpureum* (Pers. ex Fr.) Fr. (≡ *Chondrostereum purpureum* (Pers. ex Fr.) Pouz.) causes the so-called silver leaf disease common on plum, apple, and other fruit trees.[1,2] In Alberta it is also found on mountain ash, cotoneaster, and aspen.[3] The fungus enters through wounds, grows first in the heartwood, then kills the sapwood and bark. Infected trees develop foliage with a dull leaden or metallic lustre, thus the name silver leaf disease.[1] In this paper we report the structures of three sesquiterpenoid metabolites produced when *S. purpureum* is grown in liquid culture. These metabolites represent a new structural type among the sesquiterpenoids.

S. purpureum was grown in malt extract-dextrose-peptone liquid culture. Extraction of the culture broth with ethyl acetate provided crude metabolites which caused "silvering" in mountain ash seedlings.[3] The crude metabolites were separated into neutral and acidic fractions and the latter fraction was further separated by chromatography over silica gel to give a nicely crystalline acid $C_{15}H_{22}O_3$, m.p. 203–207°, for which we propose the name *sterpuric acid*. Esterification (diazomethane) of the remaining fractions followed by further chromatographic purification led to the isolation of two other metabolites, *hydroxysterpuric acid* ($C_{15}H_{22}O_4$) and *hydroxysterpuric acid ethylidene acetal* ($C_{17}H_{24}O_4$), in the form of their methyl esters.

In order to facilitate the discussion, the chemistry of sterpuric acid, which led to the derivation of the



1 R = R′ = H
2 R = CH_3, R′ = H
3 R = CH_3, R′ = $COCH_3$



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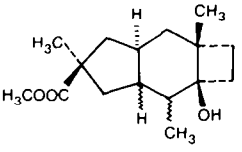

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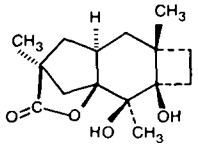

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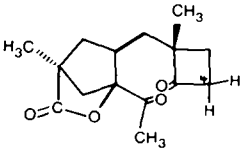

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†Dedicated to the memory of Professor R. B. Woodward.
Presented in part at the 63rd Canadian Chemical Conference, Ottawa, June 1980. Abstracts, p. 120.

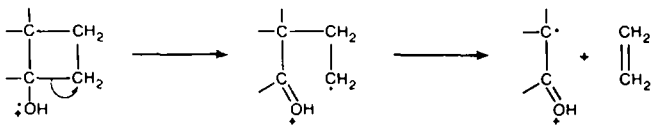
complete structure, is presented in terms of the final structure **1**.‡ This structure has been confirmed by an X-ray crystallographic study which is presented subsequently. Sterpuric acid readily forms a methyl ester (**2**) when treated with diazomethane. The tertiary (and hindered) nature of the OH group is reflected by the fact that formation of methyl O-acetylsterpurate (**3**) requires treatment of **2** with acetic anhydride pyridine at room temperature for 16 days (3 days in the presence of 4-N,N-dimethylaminopyridine). The remaining functionality of sterpuric acid (2 quaternary Me's, vinylic Me, fully substituted double bond), and thus its tricyclic nature, was apparent from examination of the ^1H and ^{13}C NMR spectra of **1**, **2** and **3**. The mass spectra of sterpuric acid and its derivatives all display a striking peak at $M^+ - 28$, shown by high resolution measurements to be due to the loss of ethylene, at first suggestive of a retro-Diels–Alder fragmentation in a β-maaliene-type (**4**) tricyclic sesquiterpene.[4] However, methyl dihydrosterpurate (**5**), prepared by catalytic hydrogenation of **2**, also shows a strong $M^+ - 28$ peak. At this point it was apparent that sterpuric acid possesses a carbon skeleton different from that of any of the known sesquiterpenes.

The carbon skeleton was first deduced in the following way. The 400 MHz ^1H NMR spectrum of sterpuric acid, analyzed in detail in the Experimental, shows the presence of an isolated allylic methylene group (C-11), an allylic methine proton (C-8) coupled to two otherwise isolated methylene groups (C-7 and C-9), along with an isolated 4-spin system corresponding to two methylene groups (C-4 and C-5). Assuming that the loss of ethylene in the mass spectra could be explained by cleavage of a cyclobutanol as shown in Scheme 1, and that sterpuric acid is derived without rearrangement from farnesyl pyrophosphate (**6** in Scheme 2) we arrived at the carbon skeleton **7** (Scheme 2) for sterpuric acid. At this stage it was not possible to say which of the starred Me groups is the carboxyl group of sterpuric acid.

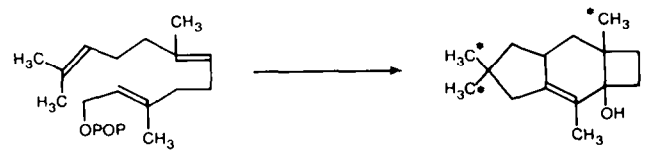
Treatment of methyl sterpurate (**2**) with osmium tetroxide in warm pyridine followed by sodium bisulfite led directly to the γ-lactone diol **8** (IR 3600, 1775 cm^{-1}), presumably formed by spontaneous lactonization of the intermediate triol. The Me group at C-2 appears as a singlet at δ 1.48 in **8**. Periodate cleavage of diol **8** gave the cyclobutanone **9**, absorbing at 1790 cm^{-1} (cyclobutanone), 1775 (γ-lactone), and 1715 (methyl ketone) in the IR. The Me group at C-2, now part of a methyl ketone, appears at δ 2.35 in the ^1H NMR spectrum. The methylene group at C-4 appears as a triplet at δ 3.00, characteristic of a cyclobutanone α-methylene.[5] The formation of **9** provided the first direct evidence for the presence of a 4-membered ring in sterpuric acid. These experiments also serve to locate the carboxyl group at C-10 rather than at C-6.

Ozonolysis of methyl sterpurate (**2**) proceeded with the uptake of two atoms of O. The product, however, displays only one CO absorption (1745 cm^{-1}) in the IR. The ^1H NMR spectrum reveals that the olefinic Me group is no longer present as such, but there is no signal for a methyl ketone. Instead, a Me signal appears at δ 1.39. The ozonolysis product is believed to possess structure **10**, arising from the expected diketone **11** by double hemiacetal formation as indicated by the arrows in **11**. In order to prevent this side reaction the ozonolysis was carried out on methyl O-acetylsterpurate (**3**) and the diketone **11** (as its O-acetyl derivative) was obtained. The cleavage product shows CO absorption at 1736 and 1710 cm^{-1} in the IR and a methyl ketone signal (δ 2.12) in the ^1H NMR spectrum.

The assignment of the stereochemistry in sterpuric acid was based on the results of the following experiments. Treatment of methyl sterpurate with *m*-chloroperbenzoic acid in CH_2Cl_2 provided the epoxide **12**, the *cis*-relationship of the epoxide and the tertiary OH group being assigned on the basis of the known preference for allylic alcohols to undergo *cis*-epoxidation.[6] Treatment of the epoxide **12** with *p*-toluenesulfonic acid in benzene brought about smooth rearrangement of the 1-hydroxybicyclo[4.2.0]octane derivative to the bicyclo[3.2.1]octan-8-one **13** *with concurrent γ-lactone formation*. The facile lactone formation indicates that the epoxide in **12**, and thus the OH in **2**, is *syn* to the carbomethoxy group. Saponification of the ester **12** gave the corresponding acid **14** without lactone formation, again in agreement with the *syn*-relationship of the carboxyl and epoxide functions. Treatment of the acid **14** with *p*-

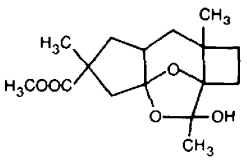


Scheme 1



Scheme 2

‡The numbering reflects the fact that sterpuric acid is a derivative of tricyclo[6,3,0,$0^{3,6}$]undec-1-ene.



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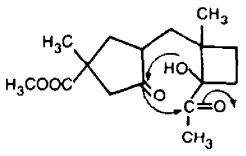

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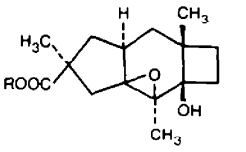
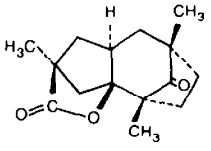


12 R = CH_3
14 R = H



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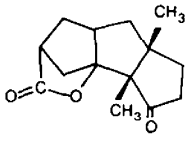

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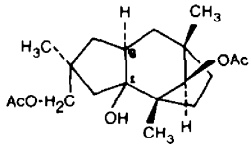

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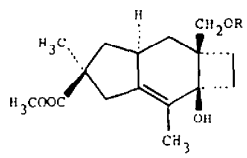
toluenesulfonic acid in chloroform provided the rearrangement product **13**. Evidence that the rearrangement of **12** proceeds with migration of the C-4 to C-3 bond rather than the C-6 to C-3 bond (to give the bicyclo[3.3.0]octanane **15**) was provided by LAH reduction of **13** to the corresponding triol followed by acetylation to give **16**. The proton geminal to the secondary acetoxyl group gives rise to a *sharp singlet* in the ^{1}H NMR spectrum, thus ruling out structure **15** for the rearrangement product.

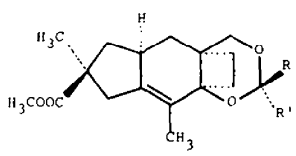
The *cis*-relationship of the C-3 OH to the C-6 Me was first suggested by the large pyridine shift[7] ($\delta_{CDCl_3} - \delta_{Py} = -0.15$ ppm) observed for this Me group in the ^{1}H NMR spectra of methyl sterpurate. It is interesting to note that the vinylic Me, which is also proximate to the OH group, likewise undergoes a substantial (−0.18 ppm) pyridine shift. The *cis*-relationship of the C-3 and C-6 substituents is further confirmed by the chemistry of hydroxysterpuric acid (see below). The configuration at C-8 was assigned on the basis of a pyridine shift study on the hydroxydiacetate **16**. The proton at C-8 shows the same chemical shift (δ 1.90) in pyridine as in $CDCl_3$, indicating that it is *trans* to the C-1 OH group, and thus *anti* to the carboxyl group in sterpuric acid. The results of the X-ray crystallographic study described below place these chemical deductions on a firm basis.

Methyl hydroxysterpurate, the ester of the second metabolite isolated, is assigned structure **17** on the basis of the following observations. The ^{1}H NMR spectrum is very similar to that of methyl sterpurate (**2**), except that the signal for the Me group at C-6 is replaced by a signal for a two proton AB quartet at δ 3.80. Brief treatment of **17** with acetic anhydride-pyridine at room temperature affords the monoacetyl derivative **18**, the C-13 methylene group now appearing as a singlet at δ 4.30. Methyl hydroxysterpurate (**17**) rapidly forms an acetonide (**19**) when treated with 2,2-dimethoxypropane under acid catalysis, confirming the 1,3-relationship of the two hydroxyl groups and the *cis*-nature of the ring fusion.

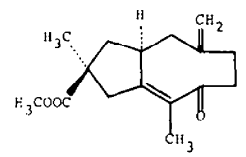
Attempts to transform the hydroxymethyl group of **17** to a Me group were unsuccessful, mainly because of the tendency of the hydroxymethylcyclobutanol system to undergo ring cleavage. Tosylation (sodium hydride, ether, *p*-toluenesulfonyl chloride)[8] of methyl hydroxysterpurate at −10° followed by room temperature work-up and chromatography provided the tosylate **20**, along with small amounts of the fragmentation product **21**. Reactions designed to bring about replacement of the tosyloxy group with hydrogen invariably led to fragmentation. Thus, treatment of **20** with sodium iodide and Zn dust in HMPA[9] gave **21** as the only isolable product. Similarly, attempts to transform the tosylate to the bromide[10] gave only **21**, and LAH reduction provided the allylic alcohol from reduction of **21**. Due to the paucity of starting material, the direct



17 R = H
18 R = Ac
20 R = Ts



19 R = R' = CH_3
22 R = CH_3, R' = H
23 R = H, R' = CH_3



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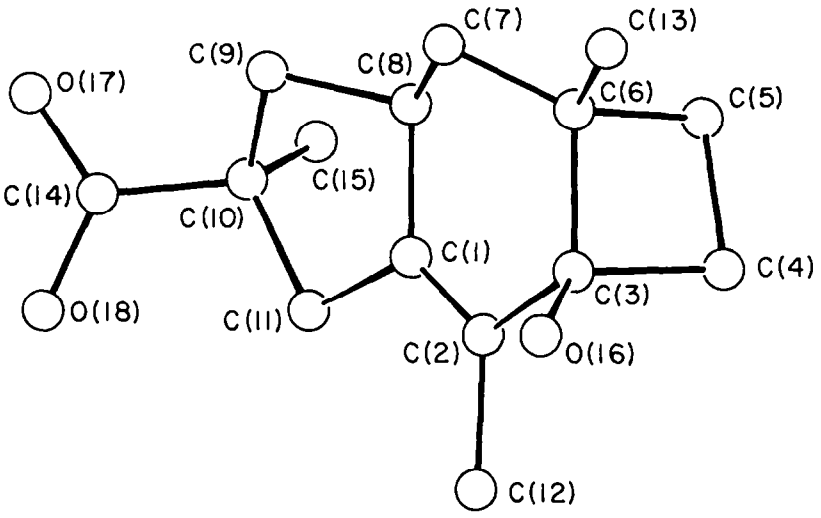

Fig. 1. A computer generated perspective drawing of sterpuric acid. Hydrogens have been omitted for clarity and no absolute configuration is implied.

correlation of methyl hydroxysterpurate (**17**) with methyl sterpurate (**2**) was not further pursued.

The third metabolite isolated is the ethylidene acetal of methyl hydroxysterpurate (**22**). The compound shows very similar spectral properties to those of the isopropylidene derivative **19**, except that the two Me singlets of the *gem*-dimethyl group of **19** are replaced by a Me doublet at δ 1.30 and a one proton quartet at δ 5.05. Acid hydrolysis of the acetal grouping in **22** gave methyl hydroxysterpurate **17**. Treatment of **17** with acetaldehyde in the presence of *p*-toluenesulfonic acid gave a *ca* 1:1 mixture of the two epimeric ethylidene acetals **22** and **23**. The two acetals were separable by repeated chromatography. Since the unnatural acetal shows both the Me signal (δ 1.25) and the methine signal (δ 4.57) at higher field than those of the natural epimer, the two are tentatively assigned the stereochemistry shown (Me group axial in **22**, equatorial in **23**).[11] The fact that only one of the epimers is isolated from the natural source, and both are formed during the laboratory preparation, suggests that **22** is not an artefact produced during the isolation process.

Sterpuric acid (**2**) and its congeners represents a new structural type among the sesquiterpenes. Interestingly, an intermediate of this carbon skeleton was proposed[12] as a possible biogenetic precursor to the isolactarane skeleton, but the biogenetic route was not seriously considered since the carbon skeleton was not among those known at the time. In order to put the structural hypothesis on a firm basis, an X-ray crystallographic study of sterpuric acid has been carried out. Figure 1 is a computer generated perspective drawing of the final X-ray model of sterpuric acid. Both of the independent molecules in the asymmetric unit have the same structure and conformation and only one molecule is shown. The X-ray experiment defined only the relative stereostructure and the enantiomer shown represents an arbitrary choice. The relative stereochemistry can be designated as 3(R*), 6(S*), 8(R*) and 10(S*). The carboxyl, OH and Me group at C-6 are all on the same face and the bridgehead hydrogen at C-8 and the Me at C-15 are on the opposite face. The cyclopentane ring has the envelope (Cs) conformation with C-9 serving as the flap; the cyclohexene ring has the 1,2-diplanar conformation with 0° torsional angles about C(1)–C(2) and C(2)–C(3) and the cyclobutane ring is puckered. In general bond distances and angles agree with commonly accepted values. The two independent molecules do not form a H-bonded carboxylic acid dimer; the closest intermolecular contacts are H-bonds between O(16)–O(18'), 2.63 Å and O(16')–O(18), 2.66 Å.

Biosynthetic studies designed to test whether the sterpuric acid skeleton is derived directly from farnesyl pyrophosphate (as outlined in Scheme 2), or *via* the protoilludane pathway,[12] are in progress.

EXPERIMENTAL

General. Mass spectra were recorded on an AEI model MS-50 mass spectrometer. The formulas of all peaks reported were determined by high resolution measurements. IR spectra were recorded on a Nicolet 7199 F.T. interferometer. Optical rotations were measured on a Perkin–Elmer model 141 automatic polarimeter. ^{1}H NMR spectra were determined on a Varian HA-100, or Bruker WH 200, or Bruker WH 400 spectrometer with TMS as internal standard. Complete spectra are reported for compounds **1**, **2**, **17** and **22**, otherwise only diagnostically significant peaks are given. Melting points were recorded on a Fisher–Johns m.p. apparatus and are uncorrected. Skellysolve B refers to Skelly Oil Company light petroleum, b.p. 62–70°. Column chromatography was carried out using Machery Nagel Kieselgel 60 (100 g/g substrate). Preparative layer chromatography (plc) was carried out on silica gel G (E. Merck, 0.75 mm layers) containing 1% electronic phosphor (General Electric, Cleveland). All solvents were distilled prior to use. All compounds for which spectral data are reported showed single spots on tlc using at least two different solvent systems.

Growth of Stereum purpureum *and extraction of metabolites.* Slant tubes of *S. purpureum* (strain C-663) were obtained from Dr. Y. Hiratsuka, Northern Forest Research Centre, Edmonton, and were maintained at 4° on potato dextrose agar. The fungus was grown in still culture at room temp on an aq. liquid medium containing malt extract (2.5%), dextrose (1.3%) and peptone (0.07%) in ten 2.8 l Fernbach

flasks containing 1 l medium per flask. After 30 days growth the mycelium was removed by filtration and the broth was concentrated at red. pres. to 1.5 l. The concentrate was extracted with ether (3 × 1.5 l) and then with EtOAc (2 × 1.5 l). The ether extract was washed with water, then brine, dried (Na_2SO_4) and evaporated to give a viscous light brown oil (1.29 g). The EtOAc extract was worked up similarly to give a dark brown oil (0.4 g).

Isolation of sterpuric acid (**1**). The ether extract from above (1.25 g) was dissolved in EtOAc (100 mL) and extracted with 10% $NaHCO_3$. The organic phase was washed with water, then brine, dried and concentrated to provide neutral material (0.83 g)† as a yellow oil. The bicarbonate extract was washed twice with $CHCl_3$ (25 mL), then cooled with ice and acidified to pH 3 with 6M HCl, and extracted with $CHCl_3$ (3 × 50 mL). The $CHCl_3$ extract was washed with water, dried and evaporated to provide a viscous oil (0.34 g). The crude acids were chromatographed over silica gel. Elution with 7.5–12.5% acetone in benzene gave crude sterpuric acid (28 mg). Repeated crystallization from EtOAc provided *sterpuric acid* (**1**) as colorless crystals, m.p. 203–207°, $[\alpha]_D^{25}$ + 72° (MeOH; *c* 0.03). IR spectrum (film): 3020–3600, 1700 cm^{-1}. MS (probe, 150°) 70 eV *m/e* (rel. int.): $C_{15}H_{22}O_3$ [M$^+$, calcd: 250.1569; found 250.1573] (30), $C_{13}H_{18}O_3$ (100), $C_{12}H_{15}O$ (49), $C_{11}H_{13}O$ (49), $C_9H_{12}O$ (53), C_7H_7 (25). ^{1}H NMR (CD_3OD) (400 MHz): δ 2.86 (1 H on C-11, d, J_{gem} 17 Hz), 2.61 (1 H, C-8, br. mult., coupled to C-7 and C-9 methylenes (see below)), 2.25 (1 H on C-11, d, J_{gem} 17 Hz), 2.20 (1 H on C-4, complex mult.), 1.92 (1 H on C-4, complex mult.), 1.86 (1 H on C-9, dd, J_{gem} 12 Hz, $J_{8,9}$ 7 Hz), 1.64 (1 H on C-9, J_{gem} 12 Hz, $J_{8,9}$ 11 Hz), 1.62 (3 H, C-12, s), 1.58 (1 H on C-7, dd, J_{gem} 13 Hz, $J_{7,8}$ 6 Hz), 1.56 (1 H on C-5, complex mult.), 1.32 (3 H, C-15, s), 1.23 (1 H on C-5, complex mult.), 1.20 (3 H, C-13, s), 0.88 (1 H on C-7, dd, J_{gem} 13 Hz, $J_{7,8}$ 11 Hz). The assignments of coupling partners were confirmed by double irradiation experiments.

Methyl sterpurate (**2**) Diazomethane in ether (3 ml, 0.3 M) was added to a soln of sterpuric acid (25 mg) in ether MeOH (3 ml, 1:1). After 15 min the solvents were removed to give methyl sterpurate (26 mg) as an oil which was purified by plc. IR spectrum (film): 3600, 1730 cm^{-1}. MS (probe, 200) 70 eV *m/e* (rel. int.): $C_{16}H_{24}O_3$ [M$^+$, Calc.: 264.1726; Found: 264.1720] (43), $C_{14}H_{20}O_3$ (100), $C_{14}H_{21}O$ (47), $C_{12}H_{15}O$ (64), $C_{11}H_{13}O$ (47), $C_9H_{12}O$ (48), C_7H_7 (35). ^{1}H NMR ($CDCl_3$): δ 1.22 (3 H, s), 1.35 (3 H, s), 1.67 (3 H, s), 3.70 (3 H, s). ^{1}H NMR (Py-d_5): δ 1.35 (3 H, s), 1.37 (3 H, s), 1.85 (3 H, s), 3.60 (3 H, s). ^{13}C NMR ($CDCl_3$): δ 176.6 (s), 138.3 (s), 127.8 (s), 73.4 (s), 51.9 (q), 47.1 (s), 44.3 (t), 43.9 (s), 41.3 (dd), 36.8 (d), 34.9 (dd), 34.4 (t), 25.4 (q), 23.4 (q), 22.1 (t), 12.8 (q).

Methyl O-acetyl sterpurate (**3**). To a soln of methyl **2** (11 mg) in ether (4 ml) was added Ac_2O (0.15 ml), Et_3N (0.2 ml) and 4-N,N-dimethylaminopyridine (5 mg). The soln was heated under reflux for 3 days, then diluted with ether (50 ml) and washed successively with dil. HCl, water, and brine. Evaporation of the solvent and chromatography (eluent benzene–acetone (1:19)) gave **3** (11 mg) as a colorless oil. IR spectrum (film): 1737 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 1.20 (3 H, s), 1.36 (3 H, s), 1.50 (3 H, s), 2.00 (3 H, s), 3.68 (3 H, s). *Exact mass* Calc. for $C_{18}H_{26}O_4$: 306.1831. Found: 306.1827.

Methyl dihydrosterpurate (**5**). Methyl sterpurate (13 mg) in MeOH (5 mL) was shaken under H_2 (4 kg/cm^2) for 7 days in the presence of 5% Pd-C (80 mg). Filtration, evaporation, and plc provided methyl dihydrosterpurate (5 mg) as a colorless oil. IR spectrum (film): 3600, 1730 cm^{-1}. *Exact mass* calcd for $C_{16}H_{26}O_3$: 266.1882; found, 266.1888. The base peak in the mass spectrum appeared at *m/e* 238 and corresponded to $C_{14}H_{22}O_3$.

Osmium tetroxide oxidation of methyl sterpurate (**2**). A soln of methyl sterpurate (20 mg) and OsO_4 (30 mg) in dry pyridine (2 ml) was kept at 50 for 12 hr, then cooled and a soln of $NaHSO_3$aq (0.18 g) in water (3 mL) and pyridine (2 ml) added. This was stirred at room temp for 3 hr, then diluted with water and extracted with ether (3 × 30 ml). Evaporation followed by chromatography (eluent benzene-ether (7:3)) gave **8** (7 mg) as a colorless solid, m.p. 145–150 . IR spectrum (film): 3600, 1775; ^{1}H NMR ($CDCl_3$): δ 1.23 (3 H, s), 1.26 (3 H, s), 1.48 (3 H, s). *Exact mass* Calc. for $C_{15}H_{22}O_4$: 266.1518. Found: 266.1509.

Periodate cleavage of diol **8**. Paraperiodic acid (H_5IO_6, 20 mg) was added to a soln of **8** (7 mg) in dry THF (0.5 ml) and the soln was stirred at room temp for 10 min. Addition of ether (40 ml) and work-up in the usual manner followed by chromatography (eluent benzene-ether (9:1)) gave **9** as an oil (6 mg). IR spectrum (film): 1790, 1775, 1718 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 1.17 (3 H, s), 1.34 (3 H, s), 2.35 (3 H, s), 3.00 (2 H, t, J 8.8 Hz, cyclobutanone α-methylene). *Exact mass* Calc. for $C_{15}H_{20}O_4$: 264.1361. Found: 264.1372.

Ozonolysis of metal sterpurate (**2**). A soln of methyl sterpurate (6 mg) in MeOH (1 ml) was cooled to −60 and O_3 was bubbled in until the soln remained blue. After flushing with N_2 at −60 , Me_2S (0.2 mL) was added and the mixture was allowed to come to room temp. Evaporation of the solvent left an oil which was purified by chromatography (eluent benzene-acetone (19:1)) to give **10** (6 mg) as an oil. IR spectrum (film): 3485, 1745 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.88 (3 H, s), 1.16 (3 H, s), 1.39 (3 H, s), 3.74 (3 H, s). *Exact mass* Calc. for $C_{16}H_{24}O_5$: 296.1623; Found: 296.1626.

Ozonolysis of methyl O-acetylsterpurate (**3**). Methyl O-acetylsterpurate (11 mg) was subjected to ozonolysis as described above for methyl sterpurate to give after chromatography **11** as a colorless solid (3 mg). IR spectrum (film): 1736, 1710 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 1.27 (3 H, s), 1.40 (3 H, s), 2.06 (3 H, s), 2.12 (3 H, s), 3.71 (3 H, s). The strongest peak in the mass spectrum corresponded to M$^+$ −C_2H_2O. *Exact mass* Calcd for $C_{16}H_{24}O_5$ (M$^+$ −C_2H_2O): 296.1624. Found: 296.1626.

Epoxidation of methyl sterpurate (**2**). *m*-Chloroperbenzoic acid (25 mg of 85%) was added to a soln of **2** (17 mg) in CH_2Cl_2 (0.75 mL). The mixture was kept at room temp for 1 hr, then diluted with ether (50 ml) and washed successively with sat $NaHCO_3$aq, water, and brine. Evaporation left an oil (17 mg) which was purified by chromatography (eluent benzene–ether (9:1)) to give **12** (15 mg) as a colorless oil. IR spectrum (film): 3460, 1735 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 1.07 (3 H, s), 1.33 (3 H, s), 1.38 (3 H, s), 3.68 (3 H, s). *Exact mass* Calc. for $C_{16}H_{24}O_4$: 280.1675. Found: 280.1672.

Rearrangement of epoxide **12**. *p*-Toluenesulfonic acid (5 mg) was added to a stirred soln of **12** (8 mg) in benzene (0.5 ml) at room temp. After 4 hr CH_2Cl_2 (30 ml was added and the resultant soln was washed successively with sat $NaHCO_3$aq, water (3 ×), and brine. Evaporation of the solvents and chromatography (eluent benzene-ether (9:1)) gave **13** (5 mg) as a colorless solid, m.p. 145 148 . IR spectrum (film): 1767, 1748 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 1.06 (3 H, s), 1.12 (3 H, s), 1.33 (3 H, s). *Exact mass* Calc. for $C_{15}H_{20}O_3$: 248.1412. Found: 248.1409.

Hydrolysis of epoxide **12** *and rearrangement of acid* **14**. The epoxide **12** (32 mg) was heated under reflux with 10% NaOH in MeOH–H_2O (4:1, 5 ml) for 3 hr. Most of the MeOH was removed at the pump and water (10 ml) added. The soln was washed with ether, then acidified (ice bath) with dil HCl, and extracted with $CHCl_3$ (4 × 25 ml) to give crude **14** (25 mg). A small portion of the acid was esterified (CH_2N_2) to give **12**. The remainder (24 mg) was dissolved in $CHCl_3$ and *p*-toluenesulfonic acid (5 mg) added. After 2 days at room temp the mixture was worked up as described above to give the rearrangement product **13**, identical (tlc, IR, ^{1}H NMR) with that prepared directly from **12**.

Preparation of hydroxydiacetate **16**. The product **13** (4.5 mg) was dissolved in THF (1 ml) and LAH (15 mg) added. After 20 min excess hydride was destroyed by the addition of wet ether. The usual work-up provided an oil (3.5 mg) which was dissolved in pyridine (0.5 ml) and Ac_2O (0.25 ml) added. After 48 hr at room temp ether (40 mL) was added and the soln worked up as usual. Chromatography (eluent benzene–ether (4:1)) provided **16** as a colorless oil (3 mg). IR spectrum (film): 3510, 1739 cm^{-1}. ^{1}H NMR

†A study of the neutral metabolites is presently underway.

($CDCl_3$): δ 0.92 (3 H, s), 1.05 (3 H, s), 1.07 (3 H, s), 1.9 (1 H, m, proton on C-8). 2.04 (3 H, s), 2.14 (3 H, s), 4.00 (2 H, AB quartet, J 10.5 Hz, CH_2OAc), 4.72 (1 H, s, CHOAc). *Exact mass* Calc. for $C_{17}H_{26}O_3$ [M^+ –HOAc]: 278.1881. Found: 278.1881. The electron impact MS did not show the molecular ion, but the chemical ionization MS indicated MW 338 ($C_{19}H_{30}O_5$).

Isolation of methyl hydroxysterpurate (**17**). The crude acidic metabolites remaining after removal of sterpuric acid (0.7 g) were dissolved in ether MeOH (2:1, 30 ml) and treated with excess ethereal CH_2N_2. Evaporation of the solvents and chromatography (eluent benzene–ether (7:3)) gave crude methyl hydroxysterpurate (0.125 g) which was further purified by plc (triple elution with ether) and further column chromatography (eluent $CHCl_3$–MeOH (99:1)) to give *methyl hydroxysterpurate* (46 mg) as a colorless oil. IR spectrum (film): 3300, 1731 cm^{-1}. MS (probe 150) 70 eV *m/e* (rel. int.): $C_{16}H_{24}O_4$ [M^+, Calc.: 280.1675. Found: 280.1673] (21), $C_{14}H_{20}O_4$ (52), $C_{15}H_{22}O_3$ (52), $C_{15}H_{22}O_3$ (27), $C_{12}H_{15}O$ (47), $C_{12}H_{14}O$ (100), $C_{11}H_{13}O$ (28), C_7H_7 (21). ^1H NMR ($CDCl_3$): δ 3.80 (2 H, C-13, ABq, J_{gem} 12 Hz), 3.70 (3 H, s, ester Me), 2.86 (1 H on C-11, d, J_{gem} 17 Hz), 2.63 (1 H, C-8, br m), 2.28 (1 H on C-11, d, J_{gem} 17 Hz), 2.23 (1 H on C-4, complex mult.), 2.02 (1 H on C-4, complex mult.), 1.90 (1 H on C-9, dd, J_{gem} 13 Hz, $J_{8,9}$ 7 Hz), 1.72 (1 H on C-9, dd, J_{gem} 13 Hz, $J_{8,9}$ 12 Hz), 1.65 (3 H, C-12, s), 1.51 (1 H on C-7, dd, J_{gem} 13 Hz, $J_{7,8}$ 7 Hz), 1.48 (2 H, C-5, complex mult.), 1.36 (3 H, C-15, s), 1.20 (1 H on C-7, dd, J_{gem} 13 Hz, $J_{7,8}$ 11 Hz). The assignments of coupling partners were verified by double irradiation experiments.

Acetylation of methyl hydroxysterpurate (**17**). Ac_2O (0.25 ml) was added to a soln of **17** (15 mg) in pyridine (0.5 ml) and the soln kept at room temp for 1.5 hr. After removal of the solvents the crude product was chromatographed (eluent benzene–ether: (9:1)) to give **18** (13 mg) as a colorless oil (13 mg). IR spectrum (film): 3340, 1735 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.34 (3 H, s), 1.65 (3 H, s), 2.08 (3 H, s), 3.68 (3 H, s), 4.31 (2 H, s). *Exact mass* Calc. for $C_{18}H_{26}O_5$: 322.1780. Found: 322.1791.

Methyl hydroxysterpurate acetonide (**19**). Compound **19** (15 mg) was dissolved in 2,2-dimethoxypropane (2 ml) and a catalytic amount of *p*-toluenesulfonic acid added. After 1 hr at room temp ether (40 ml) was added and the soln was washed with sat $NaHCO_3$aq. Evaporation of the solvent and purification by chromatography gave the **19** as an oil (15 mg). IR spectrum (film): 1731 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.26 (3 H, s), 1.36 (3 H, s), 1.39 (3 H, s), 1.59 (3 H, s), 3.00 (2 H, AB q, J 12 Hz), 3.70 (3 H, s). *Exact mass* Calc. for $C_{19}H_{28}O_4$: 320.1988. Found: 320.1994.

Methyl hydroxysterpurate p-toluenesulfonate (**20**). NaH (12 mg) was added to a soln of methyl hydroxysterpurate (30 mg) in ether (2 ml) and the mixture stirred at room temp for 12 hr. The soln was then cooled to −10° and *p*-toluenesulfonyl chloride (22 mg) in ether (1 ml) added. Stirring was continued 1 hr at −10°, then 1 hr at room temp. Brine (5 ml) was added and the mixture extracted with ether (3 × 20 ml). Evaporation of solvent and chromatography (eluent benzene–ether (19:1)) gave **20** as an oil (20 mg). ^1H NMR ($CDCl_3$): δ 1.33 (3 H, s), 1.60 (3 H, s), 2.45 (3 H, s), 3.70 (3 H, s), 4.21 (2 H, AB q, J 9 Hz), 7.33 (2 H, d, J 8 Hz), 7.79 (2 H, d, J 8 Hz). The highest peak in the MS corresponded to M$^+$ –H_2O. *Exact mass* Calc. for $C_{23}H_{29}O_5{}^{32}S$: 416.1657. Found: 416.1659. Earlier fractions from the chromatography gave the cleavage product **21** (3 mg) described below.

Attempted transformation of **20** *to methyl sterpurate* (**2**). The *p*-toluenesulfonate **20** (7 mg) was dissolved in HMPA (2 ml) and NaI (19 mg) and Zn dust (17 mg) were added. The mixture was heated at 105° for 3 hr, cooled, and diluted with ether (40 ml). Work-up in the usual manner and purification by plc gave the fragmentation product **21** (3 mg) as a colorless oil. IR spectrum (film): 1730, 1650 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.28 (3 H, s), 1.82 (3 H, s), 3.72 (3 H, s), 4.63 (2 H, br, exocyclic methylene). *Exact mass* Calc. for $C_{16}H_{22}O_3$: 262.1569. Found: 262.1572.

Isolation of methyl hydroxysterpurate ethylidene acetal (**22**). Fractions from chromatography of the crude acidic metabolites (total of 1.5 g crude acids, see isolation of sterpuric acid) eluted with 2.5% to 5% acetone in benzene gave solid material (43 mg) which was shown by tlc to be impure. Esterification of this material (CH_2N_2) and purification by plc (eluent, benzene–ether (3:1)) gave an oil (15 mg) which was further purified by column chromatography (eluent Skellysolve B-ether (9:1)) to give *methyl hydroxysterpurate ethylidene acetal* (**22**) as a colorless oil (12 mg). IR spectrum (film): 1731 cm^{-1}. ^1H NMR ($CDCl_3$): δ 5.05 (1 H (acetal proton), q, J 5 Hz), 4.00 (2 H, C-13, AB q, J 12 Hz), 3.70 (3 H, s, ester methyl), 2.92 (1 H on C-11, d, J_{gem} 17 Hz), 2.75 (1 H, dd, J's 9.5 and 10.5 Hz), 2.58 (1 H, C-8, br), 2.24 (1 H on C-11, d, J_{gem} 17 Hz), 1.98–1.70 (3 H, complex), 1.60 (3 H, C-12, s), 1.52–1.42 (3 H, complex), 1.34 (3 H, C-15, s), 1.30 (3 H (acetal Me), d, J 5 Hz), 1.20 (1 H, dd, J's 9 and 10 Hz). MS (probe, 150), 70 eV *m/e* (rel. int.): $C_{18}H_{26}O_4$ [M^+ Calc.: 306.1831. Found: 306.1830] (16), $C_{16}H_{22}O_4$ (18), $C_{12}H_{15}O$ (31), $C_{12}H_{14}O$ (100), C_8H_9O (27).

Methyl hydroxysterpurate (**17**) *from acetal* **22**. The acetal **22** (7 mg) was dissolved in THF (0.5 ml) and 3 M HCl (0.5 ml) added. After 3 hr at room temp ether (30 ml) was added and the soln worked up in the usual manner to give an oil (6 mg) which was purified by chromatography (eluent benzene–ether (4:1)) to give **17** (4 mg) identical (tlc, IR, ^1H NMR) with an authentic sample.

Preparation of ethylidene acetals **22** *and* **23**. A soln of metal hydroxysterpurate (30 mg), acetaldehyde (1.7 ml), and *p*-toluenesulfonic acid (1 mg) was stirred at room temp for 1 hr, then the soln was evaporated and the residue taken up in ether (50 ml). Work-up in the usual manner followed by chromatography (eluent Skellysolve B-ether (97:3)) gave a mixture of **22**, **23** and acetaldehyde trimer. This mixture was separated by repeated plc (eluent Skellysolve B-ether (4:1), triple elution) to give the unnatural epimer **23** (3.5 mg), and the natural epimer **22** (4.3 mg), both as colorless oils. The natural epimer **22** was identical (IR, ^1H NMR) with authentic material. The unnatural epimer **23** shows the following properties: IR spectrum (film): 1731 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.25 (3 H (acetal Me), d, J 5 Hz), 1.33 (3 H, s), 1.65 (3 H, s), 3.70 (3 H, s), 3.80 (2 H, AB q, J 10.5 Hz), 4.57 (1 H (acetal proton), q, J 5 Hz). *Exact mass* Calc. for $C_{18}H_{26}O_4$: 306.1831. Found: 306.1824.

Single crystal X-ray diffraction analysis of sterpuric acid (**1**). An irregularly shaped crystal of sterpuric acid crystallized from EtOAc was chosen for X-ray analysis. Preliminary x-ray photographs showed only triclinic symmetry. Precise lattice constants of $\mathbf{a} = 6.672$ (1), $\mathbf{b} = 10.446$ (2), $\mathbf{c} = 11.113$ (2) Å, $\alpha = 96.36$ (1), $\beta = 109.00$ (2) and $\gamma = 72.52$ (1) were obtained by a least-squares fit of fifteen moderate 2θ-values measured on a diffractometer. The limited sample precluded a density measurement but a plausible density of 1.52 g/cm^3 was calculated assuming two molecules of $C_{15}H_{22}O_3$ per unit cell. Since sterpuric acid is known to be chiral, these observations are uniquely accommodated by space group P1 with two molecules in the asymmetric unit.

All unique diffraction maxima with $2\theta \leq 114°$ were surveyed using a variable speed, 1 ω-scan with graphite monochromated CuKα radiation (1.54178 Å). A total of 1820 reflections were surveyed in this fashion and after correction for Lorentz, polarization and background effects, 1809 (99%) were judged observed ($|F_o| \geq 3\sigma(F_o)$). No corrections were deemed necessary for absorption or decomposition.

The intensity data were converted to normalized structure factors and attempts were made to achieve a phasing model using direct methods.[13] These initial attempts ended in completely centrosymmetric solutions which contained no plausible molecular fragments. Explicit assumption of an inversion center did not alter this result. In these attempts 150 E's with $|E| \geq 1.61$ were employed. Careful examination of these revealed that planes with h = 0 and 1 had abnormally large numbers of E's with large E values. To reduce this dominance all of the E's were rescaled, the 0kl and 1kl reflections were reduced to 85% of their initial value and all others were increased to 115% of their initial value. Phase determination was repeated with these rescaled E's and a

weighted E-synthesis of the most probable solution showed two plausible, identical 11 atom fragments. The remaining H atoms were located in a subsequent F-synthesis.[14] All 44 H atoms were located on a ΔF-synthesis following isotropic block diagonal refinement of the non H atoms. Full matrix least-squares refinements with anisotropic non H atoms and isotropic H's have converged to the current crystallographic residual of 0.061 for the observed reflections. Tables of fractional coordinates, bond distances, bond angles and observed and calculated structure factors have been deposited with the Cambridge Crystallographic Data File.[15]

At least part of the difficulty in solving this structure can be attributed to the strongly centrosymmetric distribution of atoms in two dimensions. The two molecules in the asymmetric unit are related by $y' = 1.46-y$ and $z' = 1.14-z$.

Acknowledgements—The work at the University of Alberta was financially supported by the Natural Sciences and Engineering Research Council (grant NSERC-A1473) and that at Cornell University by the National Institutes of Health (grant NIH-CA-24487). To both of these agencies we extend our thanks. We also wish to thank Dr. Y. Hiratsuka, Northern Forest Research Centre, Edmonton, for bringing the silver-leaf disease problem to our attention, and for providing cultures of *S. purpureum*.

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[13]We employed the MULTAN-77 and 78 series of programs written by P. Main and M. M. Woolfson, University of York, York, England and G. Germain, Universite de Louvain, Louvain, Belgium.

[14]The following library of major crystallographic programs was employed: MULTAN, G. Germain, P. Main and M. M. Woolfson; ORFLS (locally modified), W. R. Busing, K. O. Martin and H. A. Levy, Oak Ridge National Laboratory Report ORNL-TM-305; ORFFE, W. R. Busing and H. A. Levy, Oak Ridge National Laboratory publication ORNL-59-12-3; ORTEP, C. K. Johnson, Oak Ridge National Laboratory Report ORNL-TM-3794.

[15]Tables of crystallographic data for sterpuric acid are available from the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW and from J.C.