On a new synthesis of sterpurene and the bioactivity of some related *Chondrostereum purpureum* sesquiterpene metabolites

George M. Strunz, Richard Bethell, Michael T. Dumas, and Nick Boyonoski

Abstract: Sterpurene, a sesquiterpene hydrocarbon metabolite of *Chondrostereum purpureum*, a plant pathogen and potential mycoherbicide, was synthesized by a six-step sequence, in 33% overall yield. The key steps were a thermal [4 + 2] (Diels–Alder) cycloaddition of a ketene acetal diene with a conjugated ester dienophile, and a remarkably stereoselective [2 + 2] photocycloaddition of ethylene with the resulting conjugated ketone. Several related, more highly oxygenated, metabolites isolated from culture filtrates of *C. purpureum* (cf. Ayer) were tested for toxicity to cells of a hybrid aspen, *Populus deltoides* X *nigra*. Their phytotoxicity, at concentrations as low as 10 ppm, suggests that, like the *endo*-polygalacturonase produced by the fungus, these sesquiterpenes may be partially responsible for the foliar lesions associated with infection of deciduous species by the pathogen.

Key words : *Chondrostereum purpureum*, mycoherbicide, metabolites, sterpurenes, synthesis, Diels–Alder, photocycloaddition, pyramidalization, phytotoxicity.

Résumé : Nous avons synthétisé, en six étapes et avec un rendement global de 33%, la sterpurène, un métabolite sesquiterpénique hydrocarbonique de la *Chondrostereum purpureum*, qui est un pathogène des plantes et qui a du potentiel en tant que mycoherbicide. Les étapes clés étaient une cycloaddition [4 + 2] thermale (Diels–Alder) entre un acétal céténique diénique avec un ester conjugué diénophilique, et une photocycloaddition [2 + 2] entre la cétone conjuguée résultante avec l'éthylène, qui se produit avec une stéréosélectivité remarquable. Nous avons fait plusieurs épreuves sur des métabolites apparentés plus oxygénés, isolés (d'après Ayer) de cultures de *C. purpureum*, pour évaluer leur toxicité contre les cellules de *Populus deltoides* X *nigra*. Leur phytotoxicité, à des concentrations aussi basses que 10 ppm, suggère que, tout comme l'*endo*-polygalacturonase produite par le champignon, ces sesquiterpènes seraient en partie responsables des lésions des feuilles associées à l'infection par la pathogène des espèces décidues.

Mots clés : *Chondrostereum purpureum*, mycoherbicide, métabolites, sterpurènes, synthèse, Diels–Alder, photocycloaddition, pyramidalisation, phytotoxicité.

Introduction

The fungus *Chondrostereum purpureum* (Pers. ex Fr.) Pouz. (= *Stereum purpureum* (Pers. ex Fr.) Fr.) is widespread in North American forests and fruit orchards. The organism is a pathogen of a variety of deciduous species, causing, in fruit trees, a destructive condition designated silver leaf disease (1, 2). The foliar "silvering" symptoms result from reflection of light from leaf surfaces in which the epidermal cells have separated from the palisade cells (2), and the disease can lead to death of the host tree. *Chondrostereum purpureum* can colonize coniferous wood as a saprophyte but, in contrast to its pathogenicity to hardwoods, it is not known to cause any disease of conifers (1).

Concerns about non-target effects of synthetic herbicides have led to major constraints on the use of these compounds in forestry operations, and biological control is emerging as an attractive alternative for suppressing the development of competing vegetation in the early growth stages of crop trees. During 1995 and 1996, extensive field trials were conducted by Canadian Forest Service scientists and cooperators to test the efficacy of *C. purpureum* as a mycoherbicide in preventing regrowth of hardwood stump sprouts. The promising results of these trials have kindled our interest in examining chemical aspects of the bioactivity of this pathogen.

Pioneering studies by Ayer in the early 1980s on metabolites of *C. purpureum* led to the discovery of a novel class of sesquiterpenes that embody the carbon skeleton of sterpurene, **1**, at various states of oxidation (3). The hydrocarbon **1** was found in the mycelium of *C. purpureum* (3), while sterpuric acid **2** and sterpurene-3,12,14 triol **4** were the major components of the broth extract, which also contained **3** (3, 4). In addition, the biogenetically related isolactaranes, sterepolide **5**

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This paper is dedicated to Professor William A. Ayer, champion of natural products chemistry and champion natural products chemist (!), on the occasion of his 65th birthday.

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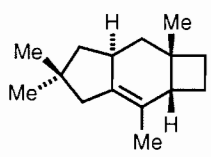

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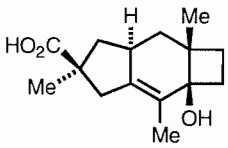

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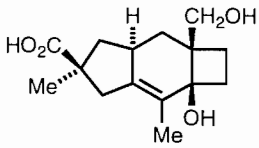

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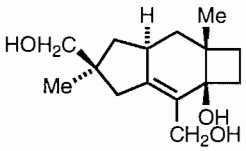

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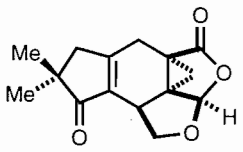

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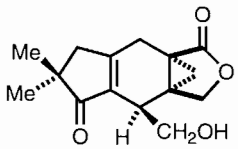

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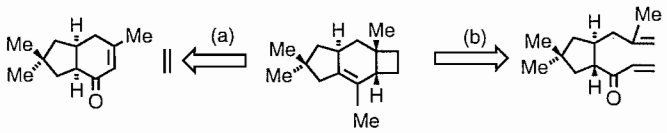
and dihydro sterepolide **6**, were produced by *C. purpureum* (5, 6). Other related metabolites of *C. purpureum* were later identified (7), and sterpurene-type sesquiterpenes were also found to be elaborated by other microorganisms (8).

Miyaire et al. (2, 9) identified an *endo*-polygalacturonase from filtrates of *C. purpureum* that induced silver leaf symptoms when injected at low doses into apple trees (~25 μg per tree). On the other hand, crude material from ethyl acetate extracts of *C. purpureum* culture broths also caused the symptoms (4), although the purified sesquiterpenes were never tested individually for phytotoxicity.[2] In an attempt to clarify chemical aspects of the interaction between pathogen and host, we reisolated a number of the sesquiterpene metabolites described by Ayer, and tested them for phytotoxicity, using cells of a hybrid aspen, *Populus deltoides* X *nigra*, for the bioassay. Another component of our research program on *C. purpureum* metabolites was the exploration and development of an efficient strategy for synthesis of sterpurene, with the potential for modification to give access to the more highly oxygenated congeners of the sesquiterpene.

The first synthesis of sterpurene, a biomimetic route from humulene, was reported in 1981 (10, cf. refs. 6, 11). This was followed by several elegant routes, each designed to exploit novel synthetic methodology (12–15).

The simplest retrosynthetic analysis, Scheme 1, suggests two formal routes, each having, as the key step, construction of a cyclobutane ring, in principle feasible by a photocycloaddition process. Inspection of models and mechanistic considerations indicated that the success of the intramolecular cyclization process (path (b) in Scheme 1), leading to simultaneous formation of six- and four-membered rings with appropriate stereochemistry, was by no means assured. Indeed, in considering products (e.g., **8**, **9**, or **9′**) that might result from photoirradiation of **7** (Scheme 2), **8** (derived by "head-to-tail" cycloaddition (16)) seemed the more probable outcome. The latter product was not, however, devoid of interest, as its reaction with methyl Grignard reagent should afford carbinol **10**,

Scheme 1.



which, on protonation, could be expected to rearrange to **11** (**11′**). An intermediate embodying the same carbon skeleton has been postulated in the biosynthesis of sterpurene (6, 11) (Scheme 2), giving us some incentive for exploring briefly the photochemistry of **7**.

The more promising intermolecular photocycloaddition strategy, path (a) (Scheme 1) was employed by Little and co-workers (12) and Paquette et al. (17) in the synthesis of sterpurene, **1**, and sterpuric acid, **2**, respectively. In the course of both these syntheses, interesting stereochemical effects were observed that seemed to warrant further investigation.

This paper reports our contributions to date in the arena of bioactivity and synthesis of *C. purpureum* metabolites.

Results and discussion

The starting material for both inter- and intramolecular photocycloaddition routes, methyl 4,4-dimethylcyclopent-1-ene carboxylate **14** (Scheme 3), was readily prepared from commercially available 4,4-dimethyl-2-cyclohexen-1-one by hydrogenation to 4,4-dimethylcyclohexanone, α-carboxymethylation (18), and chlorination. Dehydrochlorination–decarbonylation of the α-chloro-β-ketoester product, by treatment with sodium carbonate in boiling xylene according to Buchi et al. (19), gave the desired conjugated ester **14**. The overall yield of **14** from 4,4-dimethyl-2-cyclohexen-1-one (Scheme 3) was 52%, making this route a useful alternative to an earlier synthesis, via photoirradiation of diazodimedone (20).

Ester **15** was prepared in 40% (not optimized) yield by conjugate addition of isobutenyltrimethyl silane to **14**, catalyzed by tetrabutylammonium fluoride according to Majetich et al.

[2] W.A. Ayer. Personal communication.

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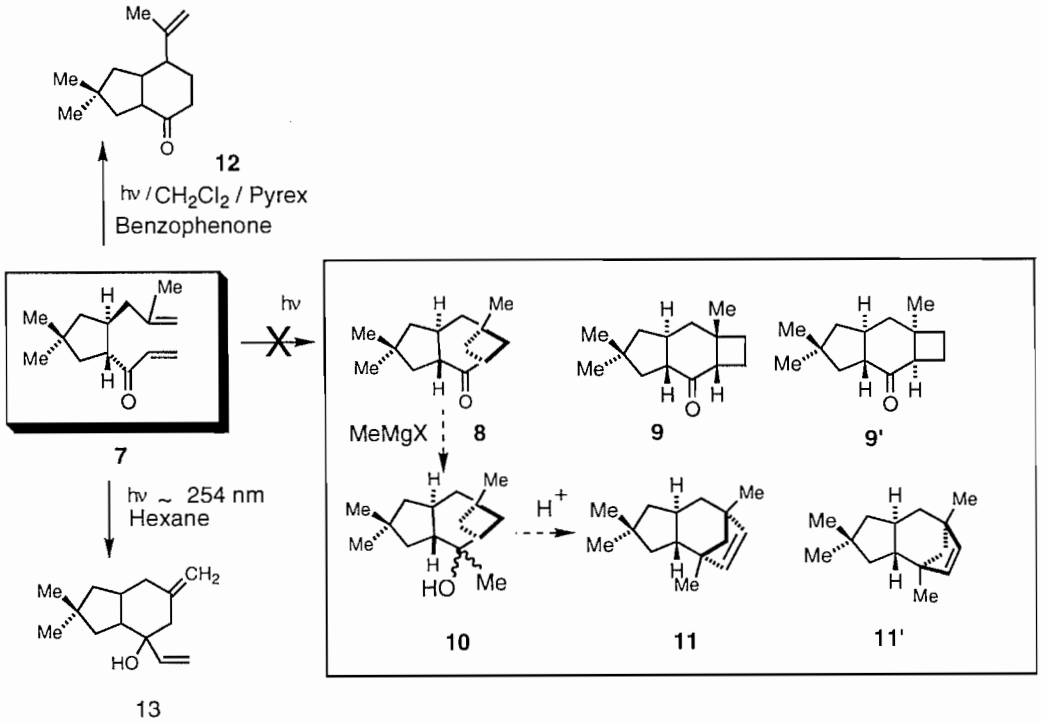
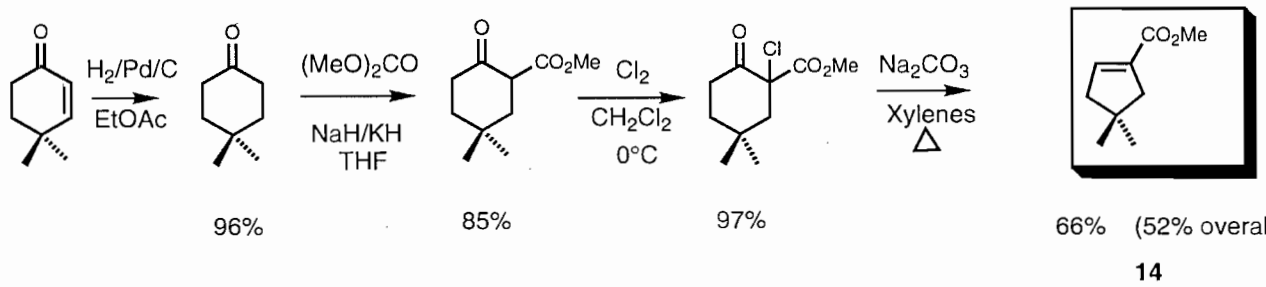
Scheme 2.



Scheme 3.



(21) (Scheme 4). (A by-product (25%) of this process was the dimeric ester product **16**, which was accessible in preparatively useful yield (~75%) when the silane was omitted. Synthetic applications of this fluoride-catalyzed dimerization and related processes are being investigated independently.) Conversion of ester **15** to the aldehyde **17** was effected in two steps, via reduction to the alcohol and reoxidation using the Swern procedure (22). Addition of vinyl magnesium bromide to the aldehyde afforded **18**, in 82% yield, and the synthesis of the desired conjugated ketone **7** (98%) was completed by Swern oxidation of the carbinol. (A minor by-product (<10%), not readily separable from **7**, was tentatively identified as the corresponding *cis* isomer.) Enone **7**, prepared thus, exhibited an ultraviolet absorption maximum (in cyclohexane solution) at 221 nm (ε 2145). The low extinction coefficient of this absorption band suggests deviation from coplanarity in the conjugated system (23).

In preliminary experiments, external irradiation (Hanovia 200 W mercury arc lamp) of a dichloromethane solution of **7** at room temperature for several hours through Pyrex (λ > 280 nm) or Corex (λ > 260 nm) filters led to recovery of unchanged starting material, (along with a small amount of presumably polymeric material, which was immobile on silica gel chromatography). Irradiation of a solution of **7** in the same solvent for 1 h at 0°C through a Vycor filter (5% transmission at 220 nm, 45% at 240 nm) led to extensive decomposition, but allowed recovery of ~4% of an isomeric product. The latter was obtained in a somewhat more satisfactory yield (39%) by photoirradiation of a dichloromethane solution of **7** at 0°C through Pyrex for 30 min, using benzophenone as sensitizer. The photoproduct was identified as **12** (Schemes 2 and 5) on the basis of its spectroscopic characteristics, especially aspects of the ^{1}H NMR spectrum. The three signals between δ 6.38 and 5.74, characteristic of the vinyl ketone, were no longer present, whereas signals at δ 4.63 and 1.65, corresponding, respectively, to the *geminal* vinyl methylene and allylic methyl hydrogens of the isopropenyl group, were retained. A multiplet at δ 2.75–2.60 could be assigned to the three protons α to the carbonyl group. Peaks attributable to a minor diastereomer were also observed (see Experimental). Infrared

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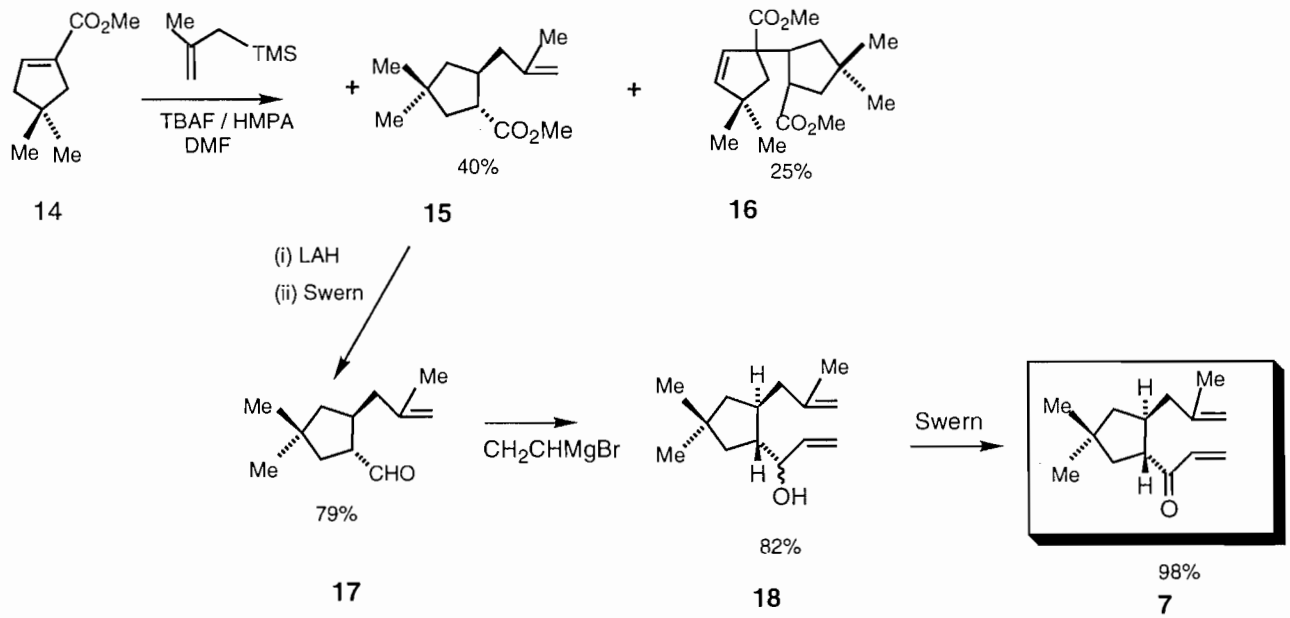
Scheme 4.

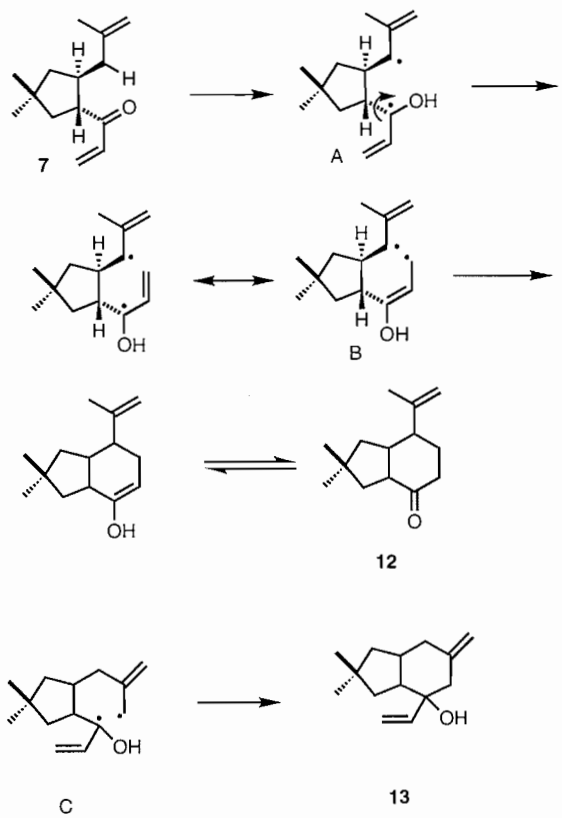


absorption at 1705 cm^{-1}, associated with the (nonconjugated) ketone group, was consistent with structure **12**.

In another experiment, a dilute hexane solution of **7** at room temperature in a quartz cell was irradiated (externally) at 254 nm for 48 h with a 4 W Spectroline E-Series UV lamp. Starting material was consumed, with concomitant formation of a complex product mixture. Separation attempts afforded material (ca. 20%) that was apparently homogeneous by TLC, but resolved by GC on a capillary column into two major products in the approximate ratio 2:3. These were shown by GC–MS to be isomers (and isomeric with the starting material), *m/z* 206. Whereas the available data were insufficient for rigorous identification of these products, they suggested the vinyl alcohol **13** and a tricyclic ketone (lacking olefinic protons) (cf. Scheme 2). Thus, the NMR spectrum of the mixture was free of signals below δ 3.0, other than a clean pattern for five vinylic protons that was fully consistent with the vinyl carbinol structure **13**. Major ions in the mass spectrum of the smaller GC peak with lower retention time supported this tentative assignment. All hydrogens of the second isomer must be assigned among the abundant NMR signals in the region above δ 3.0, not inconsistent with a tricyclic product. Significant absorptions in the infrared spectrum of the mixture are comparable to peaks in the spectrum of **29**. Ions at *m/z* 178 and 82, which are abundant in the mass spectrum of the major GC peak (and insignificant in the smaller peak), corresponding in the HREIMS of the mixture to $(M - C_2H_4)^+$ and $(C_5H_6O)^+$, can be rationalized in terms of structure **9** or **9′** (or corresponding diastereomeric structures[3] arising from photocyclization of the minor *cis* isomer (<10%) accompanying **7**) (cf. spectrum of **29**, experimental section).

A reasonable mechanism for formation of **12** from the photoirradiation of **7** might involve γ-hydrogen transfer (24, 25) producing the diradical **A**, which, after bond rotation, could cyclize to the enol form of **12**, as depicted in Scheme 5. Under different conditions, appropriate hydrogen transfer in either **7** or the diradical **A** could afford diradical **C**, suitably disposed for cyclization to **13**.

Scheme 5.



[3] A referee has pointed out that a photoproduct obtained in a yield of this magnitude could well arise not from **7**, but from the small amount of *cis* isomer accompanying it.

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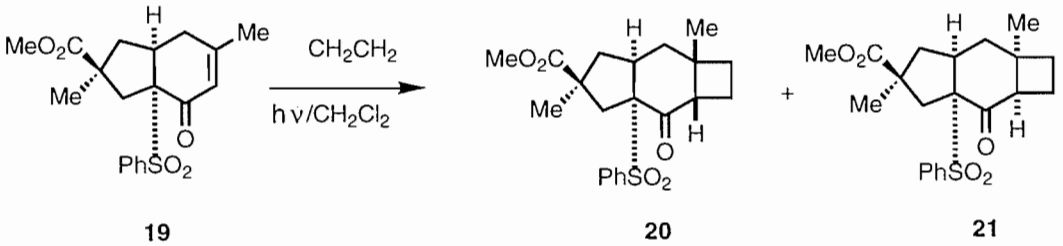
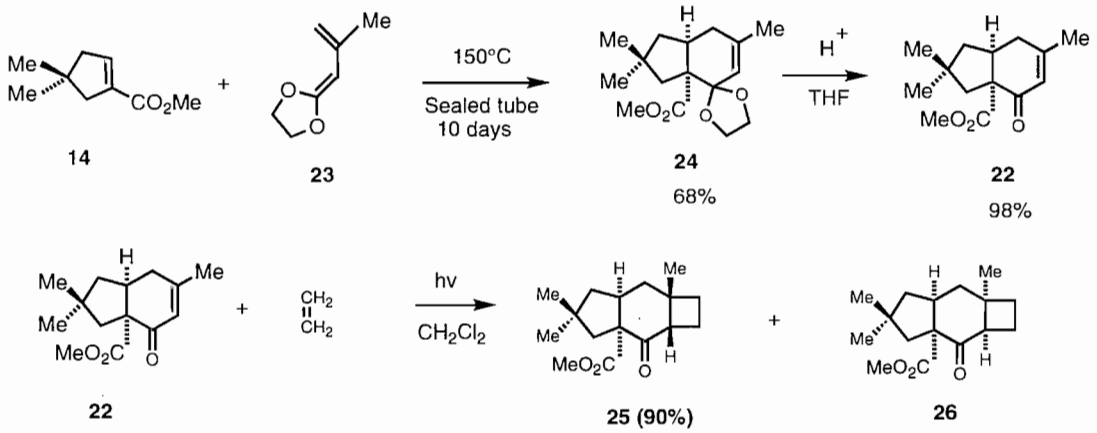
Scheme 6.



Scheme 7.



This cursory preliminary examination of the photochemical behavior of **7**, in which tricyclic product(s) were produced, if at all, in very low yield, did little to allay our reservations about path (b) (Scheme 1), and we refocussed our attention on the intermolecular photocycloaddition strategy, path (a).

In the synthesis of sterpuric acid, **2**, Paquette et al. (17) observed that photocycloaddition of ethylene to the sulfone **19** occurred with only modest stereoselectivity, affording a mixture of the desired product **20** and the diastereomeric **21,** in a ratio of 3:1 (Scheme 6). As it appeared highly probable that the sulfone and methyl ester functionalities in the *cis*-fused ring system **19** were playing a significant role in determining the stereochemical outcome of the ethylene photocycloaddition (cf. ref. 12), it was of some interest to examine the stereochemistry of photocycloaddition to the *cis*-indane-ester-enone **22** (Scheme 7), which is a logical intermediate for the synthesis of sterpurene.

Bicyclic enone **22** was readily synthesized, in 67% yield, by thermal Diels–Alder reaction of methyl 4,4-dimethylcyclopent-1-ene carboxylate **14** (prepared above) with ketene acetal **23** (26), and deprotection of the acetal product **24** (Scheme 7). Irradiation of **22** in methylene chloride solution in the presence of a large excess of ethylene at −78°C was conducted to approximately 50% conversion of enone (cf. ref. 17) using a Hanovia 200 W mercury arc lamp. The total yield of cycloaddition product, based on consumed starting material, was 97%, and it was gratifying to observe that the ratio of desired product **25** to its diastereomer **26** (Scheme 7) was 12:1 !

An empirical model for predicting stereoselectivity in photocycloaddition of olefins to cyclic α,β-unsaturated ketones was advanced by Wiesner some 20 years ago (27). This was based on the concept that "the preferred configuration of the excited state determined the configuration of the major photoadduct." It was proposed that, in the excited state, the α-carbon could be considered trigonal and that the most stable configuration of a *pyramidalized* β-carbon would determine the facial selectivity of the photoaddition (27). Notwithstanding the manifestly empirical nature of these ideas, which Wiesner was at pains to emphasize, and although the model has encountered some skepticism (28), its ability to predict stereoselectivity in photocycloadditions of olefins to enones has been impressive. Insofar as it can be applied in the present case, the stereoselectivity predicted seems to be in accord with our observed results. Thus, according to the model, the excited state of **22** may be regarded as resembling one of the conformers **A** or **B** (R = CO_2CH_3), with the methyl group in the more stable pseudoequatorial configuration (Fig. 1). Inspection of molecular models and simple MM2 calculations (29) on conformers of a formally similar (ground state) structure[4] suggest that both conformers, **A** and **B** (R = CO_2CH_3), could be present under the reaction conditions, with the former predominating. For steric reasons, reaction at the α-face of **A** (R = CO_2CH_3), giving the observed major product **25**, would then appear to be more favorable than attack at the β-face of **B** (R = CO_2CH_3) (cf. ref. 12).

More recently, attempts have been made to understand the stereoselectivity of ground state and excited state additions to enones in the context of pyramidalization of the three trigonal carbon atoms of the conjugated system, predicted on the basis of X-ray crystallographic structures and theoretical calcula-

[4] The enol of the saturated ketone corresponding to **22** was used for modeling, approximately, the Wiesner empirical representation of the excited state.

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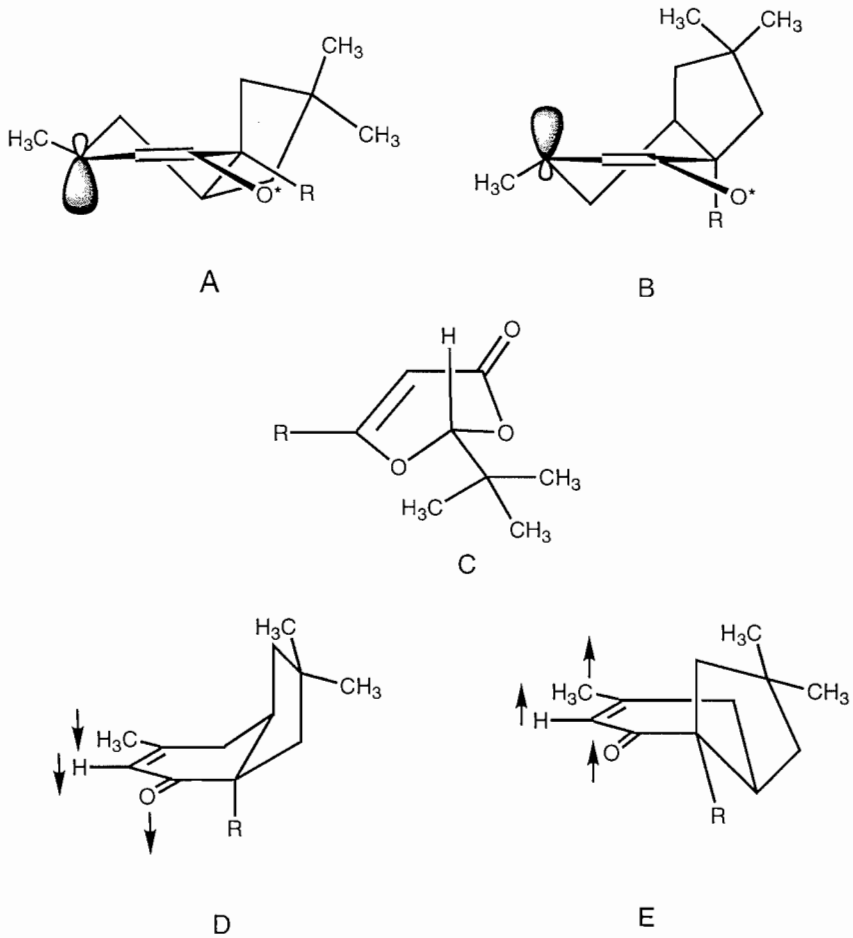
Fig. 1.



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tions. Thus, Seebach et al. (30) analyzed factors controlling or associated with facial selectivity in addition of organocuprates to (and catalytic hydrogenation of) dioxinones containing a stereogenic acetal center, e.g., **C** (Fig. 1). He noted that "the stereoselectivities of thermal and photochemical additions to dioxinones and other systems are correlated with pyramidalizations of the reacting centers," suggesting that "the steric course of attack on a trigonal center can be predicted from the direction of its pyramidalization." (30). There is strong pyramidalization in the excited state, with two of the three computed triplet states pyramidalized in the direction opposite to that in the ground state (30), which could account for the observation that ground state conjugate additions and photocycloadditions have opposite facial selectivity (31, 32).[5]

Lange and co-workers (33) reported further studies on the facial selectivity of ground state and excited state reactions of related systems, in the context of enone pyramidalization. In the course of this work, he observed that some simple cyclohexenones also displayed pyramidalization of trigonal carbons in the ground state, though to a lesser extent. It was noted that "in those systems where there is significant pyramidalization, the groups at C-4 to C-6 are always bent below the plane, i.e., away from the pucker at C-2" (33), (numbering as for 1,3-dioxin-4-one).

Simple molecular mechanics calculations (MM2* force field using MacroModel 4.5) (29) suggest that of the sofa conformations of our bicyclic enone **22**, represented approximately as **D** (R = CO_2CH_3) and **E** (R = CO_2CH_3) (Fig. 1), the former are all more stable than the latter, with an average energy difference of ~2 kcal/mol (signifying a significant preponderance of the former conformers at −78°C). In the absence of high-level calculations, an attempt to extend Lange's findings on ground state pyramidalization in simple cyclohexenones to the excited state of a system such as **D** (R = CO_2CH_3) is clearly fraught with pitfalls! It may, nevertheless, be more than coincidence that an analysis, based roughly on the observations of Lange and Seebach, appears to point to the observed facial selectivity for photocycloaddition of ethylene to **22**. Thus, the three trigonal carbon atoms of the preferred conformers **D** (R = CO_2CH_3) might be slightly pyramidalized

[5] The calculated opposite pyramidalization for enone ground and excited states (30) does not, however, appear to account adequately for the fact that facial selectivity was the *same for Diels–Alder reactions and photocycloadditions* (32). In Sato's view, cycloaddition reactions occur at the less hindered face of dioxinone enones in the sofa conformation "irrespective of the electronic states of the substrates," whereas the facial selectivity of "rather exceptional" thermal reactions occurring under kinetic control at the opposite face may be associated with pyramidalization (32).

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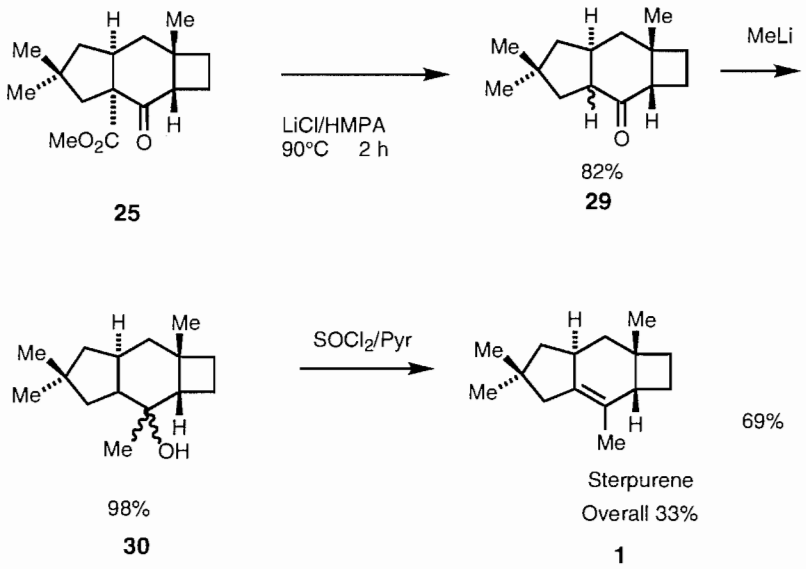
Scheme 8.

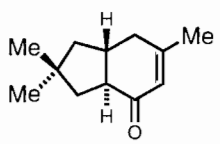


25 **29** **30** **1**

in the ground state, with displacement of the attached groups below the approximate plane formed by five of the ring carbons (away from the "pucker") as shown by the arrows (33) (Fig. 1). If, then, pyramidalization is reversed in the excited state, in accordance with Seebach's proposal (30), reaction at the bottom face of **D** (R = CO_2CH_3) would indeed be preferred.

The fact that the stereoselectivity observed by Paquette et al. (17) was somewhat lower in photocycloaddition of ethylene with **19** could be due to differences in conformation associated with the large sulfone group and the ester on the five-membered ring, and other steric effects.

Little and co-workers reported (12) that, whereas photocycloaddition of ethylene to the *trans*-fused enone **27** (lacking substitution at the ring junction) proceeded smoothly, the *cis*-fused isomer **28** *was unreactive under the same conditions*. This result is surprising. Molecular mechanics calculations indicate that the two sofa conformations of **28**, **D** (R = H) and **E** (R = H) (Fig. 1), do not differ significantly in energy. The former, which should be reactive in photocycloaddition, is more stable by ~0.3 kcal/mol. In the light of the above discussion, it does not seem obvious why **22** and **28** should differ so dramatically in their reactivity with respect to photocycloaddition.



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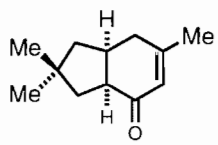

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Whatever the basis for the satisfactory stereoselectivity of the photocycloaddition of **22** with ethylene, the stage was now set for completion of the synthesis of racemic sterpurene (Scheme 8). Demethoxycarbonylation of **25**, by Krapcho's procedure, using LiCl–HMPA (34), afforded a mixture of two epimeric ketones **29** (82%) that, without separation, was treated with methyllithium to give a diastereoisomeric mixture of methyl carbinols **30** in a total yield of 98%. Finally, treatment of **30** with thionyl chloride and pyridine (12) effected dehydration, affording a 69% yield (33% overall from **14**) of racemic sterpurene **1**, whose spectra were in accord with those in the literature (3) and with data kindly supplied by Professor Ayer. (The structure of synthetic **1** and NMR assignments were confirmed by 2-D NMR experiments.)

Biological activity of some *C. purpureum* sesquiterpene metabolites

Several metabolites, previously described by Ayer et al. (3–6), were reisolated from culture filtrates of *C. purpureum*, and sterpuric acid **2**, sterepolide **5**, and dihydrosterepolide **6**, as well as synthetic sterpurene **1**, were assayed for phytotoxicity to cells of hybrid aspen, *Populus deltoides X nigra*. The results in Table 1 show that, of the compounds tested, sterpuric acid **2** is the most toxic to the aspen cells, but that the dose response curve for **2** is steeper in the concentration range studied (50–10 ppm) than for sterepolide **5** and dihydrosterepolide **6**. Sterpurene **1** was devoid of phytotoxicity to *P. deltoides X nigra* cells at the levels tested. The crude methylene chloride extract at 500 ppm exhibited approximately the same degree of phytotoxicity as sterpuric acid at 50 ppm.

Conclusions

We have synthesized the *C. purpureum* sesquiterpene metabolite, sterpurene **1** by a six-step pathway including a relatively stereoselective [2 + 2] photocycloaddition process, in an overall yield of 33% (the highest yield for any synthesis of the hydrocarbon reported to date).

Some metabolites of *C. purpureum,* previously described by Ayer, were reisolated, and bioassays of sterpuric acid **2**, sterepolide **5**, and dihydrosterepolide **6** showed them to be toxic to cells of hybrid aspen, *P. deltoides X nigra* at concentrations as low as 10 ppm. This suggests that sesquiterpene metabolites may play a role, along with the *endo*-polygalacturonase described by Miyaire et al. (2, 9), in the pathogenicity of *C. purpureum* to hardwood trees.

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Table 1: Percent viability of *Populus tremuloides* cells treated with *C. purpureum* sesquiterpenes. Values within a column followed by the same letter do not differ significantly at $p < 0.05$ (Student Neuman Keuls test).

Treatment		% Viability
Control		89.9 a
C. purpureum extract	(1000 ppm)	0 b
	(750 ppm)	10.8 c
	(500 ppm)	26.8 d
	(250 ppm)	54.2 ef
	(100 ppm)	80.3 a
Sterpuric acid, **2**	(50 ppm)	24.6 d
Sterpuric acid, **2**	(10 ppm)	61.4 f
Sterepolide, **5**	(50 ppm)	56.6 ef
Sterepolide, **5**	(10 ppm)	55.2 ef
Dihydrosterepolide, **6**	(50 ppm)	51.9 ef
Dihydrosterepolide, **6**	(10 ppm)	45.8 e
Sterpurene, **1**	(50 ppm)	90.8 a
Sterpurene, **1**	(10 ppm)	90.7 a

Experimental

General

Melting points were determined on a hot-stage apparatus and are uncorrected. Infrared spectra were recorded on a Philips Pye Unicam PU9516 infrared spectrophotometer and the ultraviolet spectra on a Philips PU 8800 UV–VIS spectrophotometer. Accurate mass measurements were made on a Kratos MS-50 instrument, which was also used for obtaining the FAB spectra. The GC–MS analysis was conducted using a HP 5989A (Engine). (The sample, in methanol solution, was injected onto a 30 m DB-1ht column (injection temperature 250°C, temperature program 60–300°C at 20°C/min). Source and transfer line temperatures were 275°C and 280°C, respectively, and data were acquired in full scan, EI mode, over the range 40–359 amu, 1.65 scans/s). NMR spectra were recorded in $CDCl_3$ on a Varian XL-200 spectrometer operating at a frequency of 200.068 MHz for ^1H, and on a Varian Unity 400 operating at a frequency of 399.946 MHz for ^1H and 100.564 MHz for ^{13}C.

2-Carbomethoxy-4,4-dimethyl-1-cyclohexanone

2-Carbomethoxy-4,4-dimethyl-1-cyclohexanone was prepared, in two steps from commercially available 4,4-dimethyl-2-cyclohexene-1-one, essentially according to Liu's procedure (35). Hydrogenation of the starting material at atmospheric pressure (101.3 kPa) (rather than at 15 psi (1 psi = 6.9 kPa) (35)), and the addition of a catalytic amount of potassium hydride with the sodium hydride in the carboxymethylation step (18), did not affect the yields (overall 82%).

2-Chloro-2-carbomethoxy-4,4-dimethyl-1-cyclohexanone

Chlorine gas was passed into a vigorously stirred solution of 2-carbomethoxy-4,4-dimethyl-1-cyclohexanone (23.5 g, 128 mmol) in freshly distilled CH_2Cl_2 maintained at 0°C. During a period of 10–20 min, color changes from pale yellow to dark green to pale orange occurred, at which time chlorine addition was discontinued. (Thin-layer chromatography indicated that all starting material had been consumed at this stage.) The mixture was purged with argon, and solvent was removed in vacuo. The crude oily residue was purified by flash chromatography on a column of silica gel, eluting with hexane–EtOAc (10:1) to furnish the oily chloro ketoester (27.0 g, 97%); IR ($CHCl_3$): 3100–2800, 1730, and 1435 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ: 3.81 (3H, s), 2.82–2.65 (3H, m), 2.07–2.00 (1H, m), 1.77–1.69 (2H, m), 1.09 (3H, s), 1.04 (3H, s); EIMS, *m/z* (relative intensity) (ion): 220 (8) $(M + 2)^+$, 219 (6) $(M + 1)^+$, 218 (18) (M^+).

Methyl 4,4-dimethyl-1-cyclopentenecarboxylate, 14 (cf. refs. 19, 20)

A solution of the chloro ketoester (315 mg, 1.44 mmol) in distilled xylenes (10 mL) was heated under reflux in an atmosphere of argon with anhydrous sodium carbonate (160 mg, 1.51 mmol) for 24 h. The mixture was cooled, filtered, and concentrated under reduced pressure. Flash chromatography of the crude residue on a silica gel column (hexane–EtOAc, 10:1) afforded 147 mg (66%) of the oily conjugated ester, **14**; IR ($CHCl_3$): 3100–2800, 1705, 1630, 1500–1400 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ: 6.64 (1H, m), 3.70 (3H, s), 2.37–2.28 (4H, m), 1.08 (6H, s); EIMS, *m/z* (relative intensity) (ion): 154 (15) (M^+), 139 (10) $(M - CH_3)^+$, 126 (34) $(M - CO)^+$, 111 (15), 95 (30) $(M - CO_2CH_3)^+$, 79 (25) , 71 (100) $(C_5H_{11})^+$, 70 (31) $C_5H_{10})^+$.

Methyl 2-(2′-methyl-2′-propenyl)-4,4-dimethylcyclopentanecarboxylate, 15

Tetrabutylammonium fluoride (trihydrate) (460 mg, 1.46 mmol: previously dried under high vacuum for 1 h) in anhydrous DMF (3.0 mL) was treated in a flame-dried flask with activated 4 Å molecular sieves at ambient temperature for 30 min. The solution was transferred to another flame-dried flask containing activated 4 Å molecular sieves, and conjugated ester **14** (1.0 g, 6.49 mmol) in anhydrous DMF (5.0 mL) was added, followed by dropwise addition of a solution of isobutenyltrimethylsilane (2.50 g, 19.5 mmol) and distilled HMPA (3.4 mL, 19.5 mmol) in anhydrous DMF (5 mL) (cf. ref. 21). The reaction mixture was stirred at ambient temperature for 1 h, when additional isobutenyltrimethylsilane (831 mg, 6.49 mmol) was added and stirring continued for 4 h. Work-up consisted of addition of methanol, then water, followed by thorough ether extraction. The combined extracts were washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. Flash chromatography of the crude product on a silica gel column (hexane–EtOAc, 10:1) afforded the desired ester **15** (550 mg, 40%) as an oil; IR ($CHCl_3$): 3100–2800, 1725, 1650, 1440, and 1370 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ: 4.63 (2H, br), 3.63 (3H, s), 2.50 (2H, m), 2.12 (1H, m), 2.0–1.88 (1H, m), 1.78–1.63 (3H, m), 1.67 (3H, s), 1.15–1.07 (1H, m), 1.02 (3H, s), 1.00 (3H,s); HREIMS, *m/z* (relative intensity) (ion): 210.1634 (10) (M^+) (calcd. for $C_{13}H_{22}O_2$: 210.1620), 195 (100) $(C_{12}H_{19}O_2)$ $(M - CH_3)^+$, 155 (24) $(C_9H_{15}O_2)$ $(M - C_4H_7)^+$, 150 (25) $(C_{11}H_{18})$ $(M - H/CO_2CH_3)^+$, 139 (36) $(C_8H_{11}O_2)$ $(M - C_5H_{11})$, 135 (39) $(C_{10}H_{15})$, 123 (11) $(C_8H_{11}O)$, 109 (19) (C_8H_{13}), 107 (10) (C_7H_7O), 95 (74) (C_7H_{11}), 93 (16) (C_7H_9).

A by-product of the reaction was the dimer **16** (350 mg), IR ($CHCl_3$) 2960, 2875, 1725, and 1480–1410 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ: 5.51 (1H, m), 5.42 (1H, m), 3.62 (6H, s),

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3.08–3.00 (1H, m), 2.68–2.63 (1H, m), 2.33–2.27 (1H, dd), 1,75–1.62 (2H, m), 1.54–1.46 (2H, m), 1.22–1.13 (1H, m), 1.11–0.99 (12 H, m); ^{13}C NMR (100 MHz, $CDCl_3$) (two stereoisomers) δ: 176.8/176.7 (s), 176.7/176.4 (s), 143.0/142.8 (d), 128.9/128.8 (d), 62.9/62.5 (s), 51.9/51.8 (q), 51.6/51.6 (q), 48.1/48.1 (d), 45.8/45.8 (t), 45.5/45.4 (d), 44.9/44.8 (s), 44.0/43.6 (t), 43.5/43.4 (t), 38.2/38.0 (s), 29.9/29.8 (q), 29.5/29.3 (q), 29.3/29.2 (q), 29.0/28.9 (q) . HREIMS, *m/z* (relative intensity) (ion): 249.1855 (100) $(M - CO_2CH_3)^+$ (calcd. for $C_{16}H_{25}O_2$: 249.1854), 233 (8) ($C_{15}H_{21}O_2$), 217 (15) ($C_{15}H_{21}O$), 189 (94) ($C_{14}H_{21}$), 173 (9) ($C_{13}H_{17}$), 154 (33) ($C_9H_{14}O_2$), 139 (20) ($C_8H_{11}O_2$).

1-Formyl-2-(2′-methyl-2′-propenyl)-4,4-dimethylcyclopentane, 17

Ester **16** (500 mg, 2.62 mmol) was added to 95% LAH (105 mg, 2.62 mmol) in anhydrous THF (15 mL) under an argon atmosphere at 0°C. The mixture was allowed to attain ambient temperature and was set aside with stirring for 3 h. Work-up consisted of addition of water (1 equiv.) followed by aqueous NaOH (1 equiv. 15% solution), and then water (3 equiv.). The mixture was filtered and the filtrates evaporated under reduced pressure. Flash chromatography of the residue on silica gel with hexane–EtOAc (10:1) as eluent afforded the corresponding amorphous alcohol (389 mg, 90%); IR ($CHCl_3$): 3625 (sharp), 3100–2800, 1650, 1480–1410, 1405–1345, and 900 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 4.67 (2H, br s), 3.66 (1H, dd, J = 10.4 and 4.7 Hz), 3.48 (1H, dd, J = 10.5 and 6.5 Hz), 2.25–2.21 (1H, m), 1.93–1.80 (3H, m), 1.72–1.57 (2H, m), 1.68 (3H, s), 1.40 (1H, s), 1.29–1.04 (2H, m), 1.00 (3H, s), and 0.98 (3H, s); EIMS, *m/z* (relative intensity) (ion): 182 (9) (M^+), 167 (52) $(M - CH_3)^+$, 164 (23) $(M - H_2O)^+$, 125 (100) $(M - C_4H_9)^+$.

Anhydrous DMSO (0.77 mL, 10.9 mmol) in freshly distilled dichloromethane (5.0 mL) was added to a solution of oxalyl chloride (0.47 mL, 5.40 mmol) in dichloromethane (20 mL) at −78°C under an argon atmosphere. After 2 min, the alcohol prepared as above (660 mg, 3.63 mmol) in dichloromethane (5 mL) was added dropwise at −78°C during a 5 min period. Stirring was continued at this temperature for 30 min and, after addition of dry triethylamine (2.52 mL, 18.1 mmol), for a further 5 min, when the mixture was allowed to warm to ambient temperature. After addition of water and dichloromethane extraction, the extracts were washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. Flash chromatography of the residue, eluting with hexane–EtOAc (10:1), afforded aldehyde **17** (625 mg, 96%) as an oil: IR ($CHCl_3$): 2950, 2870, 1715, 1650, and 1480–1400 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 9.58 (1H, d, J = 2.3 Hz), 4.67 (2H, br s), 2.53–1.71 (4H, m), 1.68 (3H,s), 1.65–1.09 (4H, m), 1.02 (3H, t), and 0.96 (3H, s); EIMS, *m/z* (relative intensity) (ion): 181 (54) ($M^+ + 1$), 180 (16) (M^+), 165 (31) $(M - CH_3)^+$, 151 (11) $(M - CHO)^+$, 136 (15) $(M - CHO - CH_3)^+$, 125 (37) $(M - C_4H_7)^+$, 124 (27) $(M - C_4H_8)^+$, 123 (77) $(M - C_4H_9)^+$ or $(M - C_3H_5O)^+$, 122 (36), 121 (18), 111 (10), 109 (27), 107 (30), and 96 (100) $(C_7H_{12})^+$.

1-(1′-Hydroxy-2′-propenyl)-2-(2″-methyl-2″-propenyl)-4,4-dimethylcyclopentane, 18

Vinyl magnesium bromide (2.0 mL 1.0 M solution in THF, 2.00 mmol) was added at 0°C under anhydrous conditions and an argon atmosphere to the aldehyde **17** (325 mg, 1.80 mmol) in freshly distilled THF (30 mL). The mixture was allowed to attain ambient temperature and was set aside with stirring for 1.5 h. After addition of saturated NH_4Cl solution, the mixture was extracted with ether, and the extracts were dried over $MgSO_4$ and concentrated in vacuo. Flash chromatography of the residue, eluting with hexane–EtOAc (10:1), afforded vinyl carbinol **18** (310 mg, 82%) as an oil; IR ($CHCl_3$) 3600 (sharp), 2950, 2940, 2865, 1650, 1600 and 1480–1400 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 5.94–5.76 (1H, m), 5.25–5.05 (2H, m), 4.65 (2H, br s), 4.14–4.00 (1H, m), 2.39–2.25 (1H, m), 2.13–1.72 (2H, m), 1.68 (3H,s), 1.65–1.03 (5H, m), and 0.98 (6H, overlapping singlets); EIMS, *m/z* (relative intensity) (ion): 208 (8) (M^+), 193 (13) $(M - CH_3)^+$, 190 (15) $(M - H_2O)^+$, 175 (24) $(M - CH_3 - H_2O)^+$, 165 (20), 152 (29) $(M - C_4H_8)^+$, or $(M - C_3H_4O)^+$, 151 (100) $(M - C_4H_9)^+$ or $(M - C_3H_5O)^+$, 147 (29) , 136 (27), and 135 (19).

1-(1′-Oxo-2′-propenyl)-2-(2″-methyl-2″-propenyl)-4,4-dimethylcyclopentane, 7

The Swern oxidation (22) of vinyl carbinol **18** was conducted as described above using oxalyl chloride (0.19 mL, 2.18 mmol), anhydrous DMSO (0.31 mL, 4.37 mmol), and triethylamine (1.0 mL, 7.2 mmol) for oxidation of the alcohol (300 mg, 1.44 mmol). The yield of product **7**, after work-up and chromatography, was 290 mg, 98%; IR ($CHCl_3$): 2950, 2865, 1690, 1670, 1610, 1450, 1405, and 1370 cm^{-1}; UV λ_{max} (cyclohexane): 221 nm (ε 2145); ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 6.38 (1H, dd, J = 10.2 and 18.1 Hz), 6.13 (1H, dd, J = 1.7 and 17.6 Hz), 5.74 (1H, dd, J = 1.7 and 10.1 Hz), 4.61 (2H, br), 2.98–2.85 (1H, m), 2.78–2.63 (1H, m), 2.12–1.44 (5H, m), 1.65 (3H, s), 1.21–1.10 (1H, m), 1.03 (3H, s), and 1.00 (3H,s); HREIMS, *m/z* (relative intensity) (ion): 191.1426 (29) $(M - CH_3)^+$, (calcd. for $C_{13}H_{19}O$: 191.1436), 173 (14) ($C_{13}H_{17}$), 138 (23) ($C_9H_{14}O$), and 95 (100) (C_7H_{11}) (in the GC–MS (total ion current), a small shoulder (<10%) on the longer retention side of the main peak, whose MS was very similar to that of **7**, is presumed to represent the corresponding *cis* isomer).

Photoirradiation of 7

(*i*) In a cylindrical Pyrex vessel, a solution of ene-enone **7** (31 mg, 0.15 mmol) in freshly distilled dichloromethane (11.0 mL) containing benzophenone (7 mg, 0.038 mmol) was purged with argon for 30 min. The solution at ~0°C was irradiated (externally) for 30 min using a Hanovia 200 W mercury arc lamp in a quartz well, the latter apparatus and the contiguous reaction vessel being cooled in an ice bath. At this stage, TLC analysis indicated that no unchanged starting material remained. The solution was allowed to attain ambient temperature, and the solvent was removed under reduced pressure. Chromatography of the product on a preparative TLC plate, eluting with hexane–EtOAc (10:1), afforded 12 mg (39%) of photoproduct **12**; IR ($CHCl_3$): 3050–2800, 1705, 1600, 1480–1400, 1390–1360, and 900 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 4.63 (2H, narrow m), 2.75–2.60 (3H, m), 2.10–1.65 (6H m), 1.65 (~0.75 Me, s), 1.54 (~0.25 Me), 1.55–1.05 (2H, m), 1.04 (s), 1.02 (s), and 0.99 (s) (6H) (peaks at δ: 1.54 and 1.04 attributed to methyl groups of minor diastereomer); LREIMS, *m/z* (relative intensity) (ion): 206 (20) (M^+), 191 (29) $(M - CH_3)^+$, 163 (43) $(M - CH_3 - C_2H_4)^+$, or $(M - CH_3 - CO)^+$, 151 (84)

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$(M - C_4H_7)^+$ or $(M - C_3H_3O)^+$, 136 (29) $(M - C_5H_{10})^+$, 123 (80) $(M - C_6H_{11})^+$, 109 (45) $(C_7H_9O)^+$, 95 (100) $(C_7H_{11})^+$, 81 (84) $(C_6H_9)^+$, 67 (25) $C_5H_7)^+$, 55 (59) $(C_3H_3O)^+$ or $(C_4H_7)^+$; HREIMS, *m/z* (relative intensity) (ion): 206.1676 (38) (M^+) (calcd. for $C_{14}H_{22}O$: 206.1671).

(*ii*) A solution of ene-enone **7** (38 mg, 0.184 mmol) in distilled hexane (3.0 mL) (0.0615 M) at ambient temperature in a quartz cell was irradiated at 254 nm for 71 h using a 4 W Spectroline E-Series UV lamp. The solution was concentrated and the residue subjected to preparative TLC on a silica gel plate (hexane–EtOAc, 10:1). Material (8 mg, ca 20%) from an apparently homogeneous band on the plate was found by GC–MS to consist of two isomeric products in the approximate ratio 2:3. The spectra of the mixture contained features in accord with structure **13**; IR ($CHCl_3$): 3600, 2940, 2870, 1690, and 1455 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ: 5.86 (1H, dd, J = 17.4 and 10.7 Hz), 5.26 (1H, dd, J = 17.2 and 1.5 Hz), 5.05 (1H, dd, J = 10.7 and 1.4 Hz), 4.86 (1H, br), 4.77 (1H,br), and 2.80–0.85 (~60 H, m); GC–MS (minor peak: (**13**) (4.41 min)) *m/z* (proposed ion): 206 $(M)^+$ $(C_{14}H_{22}O)^+$, 151 $(M - C_4H_7)^+$, 109 $(C_7H_9O)^+$, 95 $(C_7H_{11})^+$, 81 $(C_6H_9)^+$, 55 $(C_4H_7)^+$ or $(C_3H_3O)^+$; (major peak: (**9** or **9′**?) (5.12 min)) *m/z*: 206 $(M)^+$ $(C_{14}H_{22}O)^+$, 191 $(M - CH_3)^+$, 178 $(C_{12}H_{18}O)^+$, $(M - C_2H_4)^+$, 163 $(M - CH_3 - C_2H_4)^+$, 122 $(C_8H_{10}O)^+$, 95 $(C_7H_{11})^+$, 82 $(C_5H_6O)^+$, and 55 $(C_4H_7)^+$.

Diels–Alder addition of conjugated ester, 14, and ketene acetal, 23

A mixture of ester **14** (130 mg, 0.84 mmol) and ketene acetal **23** (650 mg, crude, ca. 5.15 mmol, prepared according to Boehler and Konopelski (26)) with K_2CO_3 (10 mg) was heated under argon in a sealed tube at 150°C for 10 days. Flash chromatography of the product, eluting with hexane–EtOAc (10:1), afforded the Diels–Alder adduct **24** (160 mg, 68 %); IR ($CHCl_3$): 3100–2800, 1720, and 1500–1400 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ: 5.40 (1H, s), 3.98–3.79 (4H, m), 3.67 (3H, s), 3.00 (1H, m), 2.40–2.15 (2H, m), 1.83–1.69 (5H, m), 1.63–1.52 (1H, m), 1.31–1.20 (1H, m), 1.01 (3H, s), and 0.86 (3H, s); HREIMS, *m/z* (relative intensity) (ion): 280.1668 (13) $(M)^+$ (calcd. for $C_{16}H_{24}O_4$, 280.1674), 249 (15) $(C_{15}H_{21}O_3)$ $(M - OCH_3)^+$, 221 (48) $(C_{14}H_{21}O_2)$ $(M - CO_2CH_3)^+$, 191 (14) $(C_{13}H_{19}O)$, 177 (15) $(C_{12}H_{17}O)$, 176 (14) $(C_{12}H_{16}O)$, 161 (17) $(C_{11}H_{13}O)$, 142 (13) $(C_7H_{10}O_3)$, 127 (100) $(C_7H_{11}O_2)$, 123 (16) $(C_8H_{11}O)$, 121 (12) (C_8H_9O), 111 (15) (C_6H_7O), 105 (12) (C_8H_9), 99 (22) $(C_5H_7O_2)$, 93 (11) (C_7H_9), 91 (20) (C_7H_7).

Deprotection of Diels–Alder adduct

Acetal **24** (320 mg, 1.14 mmol), from the Diels–Alder reaction, was stirred in THF (6.0 mL) containing 5% aqueous HCl (2.4 mL) at ambient temperature for 36 h. Work-up afforded the chromatographically homogeneous bicyclic enone ester **22** in a yield of 265 mg (98%); IR ($CHCl_3$): 3150–2800, 1720, 1660, and 1440 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 5.95 (1H, s), 3.65 (3H, s), 3.00–2.94 (1H, m), 2.72 (1H, dd, J = 19.3 and 6.2 Hz), 2.50 (1H, d, J = 13.7 Hz), 2.14 (1H, d, J = 19.3 Hz), 1.96 (1H, d, J = 13.8 Hz), 1.93 (3H, s), 1.58 (1H, dd, J = 12.6 and 6.8 Hz), 1.41 (1H, t, J = 13.0 Hz), 1.06 (3H, s), 0.95 (3H, s); HREIMS, *m/z* (relative intensity) (ion): 236.1399 (8) $(M)^+$, (calcd. for $C_{14}H_{20}O_3$, 236.1412), 221 (1) $(C_{13}H_{17}O_3)$ $(M - CH_3)^+$, 205 (2) $(C_{13}H_{17}O_2)$ $(M - OCH_3)^+$, 204 (2) $(C_{13}H_{16}O_2)$, 177 (14) $(C_{12}H_{17}O)$, 176 (4) $(C_{12}H_{16}O)$, 161 (6) $(C_{11}H_{13}O)$, 123 (2) $(C_8H_{11}O)$, 121 (3) (C_8H_9O), 82 (100) (C_5H_6O).

Photocycloaddition of ethylene to 22

A solution of enone ester **22** (117 mg, 0.496 mmol) in freshly distilled dichloromethane (20 mL) contained in a cylindrical Pyrex vessel was purged with argon for 30 min. As ethylene (excess) was bubbled in, the solution was irradiated (externally) for 2 h using a Hanovia 200 W mercury arc lamp in a quartz well, the latter apparatus and the contiguous reaction vessel being cooled in a Dry Ice – acetone bath. After the photocycloaddition, the solution was allowed to attain ambient temperature, and the solvent was removed under reduced pressure. Preparative thin-layer chromatography of the crude product (132 mg) on a silica gel plate, eluting with hexane–EtOAc (10:1), afforded unreacted enone ester **22** (50 mg) and 73 mg (97% based on consumed starting material) of a mixture of isomeric photoadducts **25** and **26** in the ratio (by NMR) 12:1. The isomers were separated chromatographically with the major (desired) product, **25**, eluting more slowly than its stereoisomer. Photoadduct **25**, mp 49°C, displayed the following spectroscopic properties: IR ($CHCl_3$) 3000–2800, 1730, 1690, and 1480–1400 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 3.73 (3H, s), 3.53–3.43 (1H, m), 2.75–2.71 (1H, dd, J = 10.2 and 6.6 Hz), 2.22–2.00 (5H, m), 1.90–1.85 (1H, dd, J = 13.0 and 7.7 Hz), 1.82–1.75 (2H, m), 1.41–1.36 (1H, dd, J = 13.0 and 6.7 Hz), 1.33–1.25 (1H, m), 1.22 (3H, s), 1.05 (3H, s), and 0.98 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 210.4 (s), 173.4 (s), 65.8 (s), 52.7 (q), 51.2 (d), 48.0 (t), 47.7 (t), 42.3 (d), 41.0 (s), 39.2 (t), 37.5 (s), 31.5 (q), 30.9 (t), 30.5 (q), 29.9 (q), and 17.9 (t); HREIMS, *m/z* (relative intensity) (ion): 264.1712 (8) $(M)^+$ (calcd. for $C_{16}H_{24}O_3$, 264.1725), 249 (3) $(C_{15}H_{21}O_3)$ $(M - CH_3)^+$, 233 (5) $(C_{15}H_{21}O_2)$ $(M - OCH_3)^+$, 232 (9) $(C_{15}H_{20}O_2)$, 217 (5) $(C_{14}H_{17}O_2)$, 193 (9) $(C_{12}H_{17}O_2)$, 177 (27) $(C_{12}H_{17}O)$, 155 (15) $(C_9H_{15}O_2)$, 82 (100) (C_5H_6O).

Minor photoadduct **26** displayed the following spectroscopic properties: IR ($CHCl_3$): 2960, 2875, 1705, and 1480–1405 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 3.68 (3H, s), 3.64–3.54 (1H, m), 2.75–2.71 (1H, m), 2.46 (1H, d, J = 13.8 Hz), 2.31–2.22 (1H, m), 2.16–2.07 (1H, m), 1.84–1.61 (5H, m), 1.33 (3H, m), 1.31–1.21 (1H, m), 1.15–1.09 (1H, m), 1.04 (3H, s), and 0.95 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 211.0 (s), 174.0 (s), 64.5 (s), 52.6 (q), 49.4 (t), 48.9 (d), 45.4 (t), 44.2 (d), 43.7 (s), 40.9 (t), 38.4 (s), 32.9 (t), 29.3 (q), 27.5 (q), 26.4 (q), and 17.4 (t); HREIMS, *m/z* (relative intensity) (ion): 264.1725 (18) $(M)^+$ (calcd. for $C_{16}H_{24}O_3$, 264.1725), 249 (12) $(C_{15}H_{21}O_3)$ $(M - CH_3)^+$, 233 (9) $(C_{15}H_{21}O_2)$ $(M - OCH_3)^+$, 232 (11) $(C_{15}H_{20}O_2)$, 217 (7) $(C_{14}H_{17}O_2)$, 177 (29) $(C_{12}H_{17}O)$, 176 (19) $(C_{12}H_{16}O)$, 155 (15) $(C_9H_{15}O_2)$, 123 (20) $(C_8H_{11}O)$, 110 (27) $(C_7H_{10}O)$, and 82 (100) (C_5H_6O).

Demethoxycarbonylation of 25: tricyclic ketone, 29

The major photoadduct **25** (295 mg, 1.12 mmol) dissolved in distilled HMPA (10 mL) was heated at 100°C with LiCl (95 mg, 2.24 mmol) in an argon atmosphere under anhydrous conditions for 20 h (cf. ref. 34). Work-up and flash chromatography in the normal manner afforded the desired tricyclic ketone, **29** (188 mg, 82%), IR ($CHCl_3$): 2940, 2870, 1680, 1455, and 1370 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 3.29–3.21 (1H, m), 3.13–3.06 (1H, m), 2.66–2.62 (1H, m), 2.27–

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2.14 (2H, m), 2.04–1.95 (1H, m), 1.90–1.81 (2H, m), 1.64–1.59 (3H, m), 1.16 (3H, s), 1.11–1.06 (1H, m), 1.07 (3H, s), 0.99 (3H, s), and 0.93–0.87 (1H, m); HREIMS, *m/z* (relative intensity) (ion): 206.1682 (21) $(M)^+$ (calcd. for $C_{14}H_{22}O$, 206.1671), 191 (38) $(C_{13}H_{19}O)$ $(M - CH_3)^+$, 178 (64) $(C_{12}H_{18}O)$ $(M - C_2H_4)^+$,123 (23) $(C_8H_{11}O)$, 82 (100) (C_5H_6O).

Tricyclic carbinol 30

A solution of methyllithium in ether (0.65 mL, containing 0.909 mmol) was added to tricyclic ketone **29** (170 mg, 0.825 mmol) in freshly distilled ether (10 mL) at 0°C in an atmosphere of argon under anhydrous conditions. The reaction mixture was set aside at 0°C with stirring for 10 min, after which saturated NH_4Cl solution was added. Ether extraction and standard work-up afforded, without chromatography, 179 mg (98%) of a mixture of stereoisomeric alcohols **30**; IR ($CHCl_3$): 3600 (sharp), 2940, 2870, 1690, 1455, and 1375 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ: 2.52–1.05 (13 H, m), 1.12 (3H, s), 1.07 (3H, s), 0.98 (3H, s), and 0.97 (3H, m); HREIMS, *m/z* (relative intensity) (ion): 204.1892 (15) $(M - H_2O)^+$ (calcd. for $C_{15}H_{24}$, 204.1878), 189 (9) $(C_{14}H_{21})$ $(M - H_2O - CH_3)^+$, 176 (100) $(C_{13}H_{20})$, 175 (51) $(C_{13}H_{19})$, 161 (78) $(C_{12}H_{17})$, 149 (6) $(C_{11}H_{17})$, 121 (22) (C_9H_{13}), 120 (31) (C_9H_{12}), 119 (28) (C_9H_{11}), 107 (21) (C_8H_{11}), 105 (45) (C_8H_9), 95 (5) (C_7H_{11}), and 91 (17) (C_7H_7).

Sterpurene, 1

Carbinol (mixture) **30** (39 mg, 0.176 mmol) was stirred with thionyl chloride (0.03 mL, 0.411 mmol) and dry pyridine (0.2 mL) at 0°C in an atmosphere of argon under anhydrous conditions for 1.5 h. After addition of water, the mixture was extracted with ether and worked up in the conventional manner. Flash chromatography of the crude product on a silica gel column eluting with *n*-pentane afforded pure racemic sterpurene (23.4 mg, 69%); IR ($CHCl_3$): 3025–2800, 1480–1400, and 1400–1345 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 2.63 (1H, m), 2.41–2.31 (1H, m), 2.12–2.06 (3H, m), 1.96–1.88 (1H, m), 1.68–1.64 (1H, m), 1.55–1.51 (4H, m), 1.47–1.38 (2H, m), 1.20 (3H, s), 1.11–1.05 (1H, m), 1.07 (3H, s), 1.05 (3H, s), and 0.70–0.64 (1H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 136.5 (s), 127.7 (s), 48.6 (t), 44.6 (d), 44.4 (t), 39.3 (t), 38.0 (s), 37.6 (d), 36.5 (s), 30.2 (q), 29.4 (q), 29.3 (q), 27.9 (t), 24.7 (t), and 17.8 (q); HREIMS, *m/z* (relative intensity) (ion): 204.1892 (15) $(M)^+$ (calcd. for $C_{15}H_{24}$, 204.1878), 189 (9) $(C_{14}H_{21})$ $(M - CH_3)^+$, 176 (100) $(C_{13}H_{20})$, 175 (51) $(C_{13}H_{19})$, 161 (78) $(C_{12}H_{17})$, 149 (6) $(C_{11}H_{17})$, 121 (22) (C_9H_{13}), 120 (31) (C_9H_{12}), 119 (28) (C_9H_{11}), 107 (21) (C_8H_{11}), 105 (45) (C_8H_9), 95 (5) (C_7H_{11}), and 91 (17) (C_7H_7).

Natural products

Isolation of sterpuric acid **2**, sterepolide **5**, and dihydrosterepolide **6** from culture filtrates of *C. purpureum* was previously described by Ayer et al. (3–6). Physical properties and spectroscopic characteristics of these metabolites, isolated in the present study from 30-day-old cultures, were in agreement with the literature data.

Bioassays

A callus line was initiated from a bud of *P. deltoides X nigra* (DN17) and maintained on half-strength Gamborg medium at 25°C in the dark. Ten-day-old cells were removed using a sterile bacteriological loop and approximately 5 mg (fresh weight) was added to 2 mL of the above medium (minus agar) containing the test compound dissolved in 25 μL 50% aqueous acetone. The cells were incubated in this mixture at 25°C in the dark with shaking (100 rpm) for 2 h. Viable cells could be distinguished from dead cells by their fluorescence under UV after adding fluorescein diacetate (2 μL of solution containing 5 mg/mL). For each treatment, 100 cells were examined and the ratio of living to dead cells gave the % viability.

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