Journal of Natural Products
Vol. 52, No. 5, pp. 1042-1054, Sep-Oct 1989

# ANTIFUNGAL SESQUITERPENOIDS FROM AN ARTHROCONIDIAL FUNGUS

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ABSTRACT.—The metabolites produced in liquid culture by an arthroconidial fungus have been examined, and six new hirsutane metabolites have been isolated from the antifungal neutral extract. The structures of the major metabolites, which we have named arthrosporone [**1**], anhydroarthrosporone [**2**], and arthrosporol [**3**], have been determined by chemical and spectroscopic methods.

As part of a continuing search for naturally occurring antifungal agents we have investigated the metabolites produced by an arthroconidial fungus (UAMH 4262) when grown in liquid still culture. The as yet unidentified fungus was encountered accidentally by Tsuneda and Hiratsuka as a contaminant on a culture of the phytopathogenic fungus *Ceratocystis ulmi* (Buis.) Moreau, the causative agent of Dutch elm disease (1,2). The fungus has been examined by Dr. J. Stalpers who considered it to be a haploid basidiomycete, probably belonging to a wood-inhabiting Agaricales. The sexual stage is unknown, and in culture the fungus produces an asexual stage consisting of rectangular arthroconidia with short separating cells, similar to those described as Group 3 (3). No hyphomycete genus is currently available to accommodate these basidiomycete anamorphs[1]. In competitive culture the arthroconidial fungus strongly inhibits *C. ulmi* and other wood-destroying fungi of the genus *Ceratocystis*, including those that are responsible for the blue stain disease of pine (4–6).

Fungus UAMH 4262 was grown on a medium of potato dextrose broth in still culture for approximately 30 days. Bioassays of the culture extracts revealed that the antifungal activity was located mainly in the neutral fraction of the broth extracts (7). The neutral fraction was separated into several components by Si gel chromatography. These components were further purified by Si gel chromatography and, in some instances, fractional crystallization. This led to the isolation of several new sesquiterpenoids as well as the known sesquiterpene tricyclohumuladiol (8–10). We report herein the structures of these new sesquiterpenes.

## RESULTS AND DISCUSSION

In order to facilitate the discussion, the chemistry of the arthroconidial metabolites that led to the derivation of the complete structures of these previously unknown compounds is presented in terms of the final structure of arthrosporone [**1**] and the closely related anhydroarthrosporone [**2**]. Arthrosporone ($C_{15}H_{24}O_3$, established by hrms and cims) and anhydroarthrosporone ($C_{15}H_{22}O_2$) are the most abundant crystalline metabolites and are optically active. The ir spectrum of **1** shows hydroxyl (3440 $cm^{-1}$), carbonyl (1731 $cm^{-1}$), and *gem*-dimethyl (1381, 1360 $cm^{-1}$) absorption bands. The ketonic nature of the carbonyl is apparent from its uv ($\lambda$ max 280 nm) and $^{13}C$-nmr spectra ($\delta$ 216.4) and by the fact that arthrosporone [**1**] is readily reduced with $NaBH_4$ to arthrosporol [**3**] and its epimer **4**. Both the ir and the $^{13}C$-nmr spectra suggest a cyclopentanone carbonyl.

The tricyclic nature of arthrosporone [**1**] is apparent from examination of its $^{13}C$-nmr spectrum which shows the ketonic carbonyl carbon and 14 $sp^3$ carbons: four

[1]L. Sigler, University of Alberta Microfungus Collection, personal communication, June 1989.

quaternary carbons (two of which bear an oxygen), two CH, four $CH_2$, and four Me groups (11). The $^{13}C$- and $^{1}H$-nmr spectra account for 22 hydrogens which are directly attached to carbon; thus, the remaining two hydrogens must be present as part of hydroxyl groups. The hydroxyl groups of arthrosporone [**1**] are tertiary hydroxyl groups, as revealed by examination of the $^{1}H$-nmr spectrum. There are no lowfield hydrogens (i.e., $\delta>3.0$), indicating that the molecule contains no olefinic hydrogens or hydrogens geminal to an oxygen atom. The remainder of the spectrum consists of three methyl singlets, a methyl doublet, and several well-resolved one-hydrogen spin-multiplet systems (Table 1). The lack of reactivity of **1** in oxidation reactions is consistent with the tertiary nature of its hydroxyl groups. Under forcing basic conditions ($Ac_2O$, 4-*N,N*-dimethylaminopyridine, $NEt_3$, 3 days), arthrosporone is converted to a monoacetyl derivative **5**, while acid-catalyzed acetylation provides the diacetyl derivative **6**. The ir spectrum of **5** shows hydroxyl (3449 $cm^{-1}$), carbonyl, and acetoxyl (1736, 1249 $cm^{-1}$) absorption bands. Its $^{1}H$-nmr displays acetyl methyl ($\delta$ 1.98, s) and hydroxyl hydrogen ($\delta$ 1.47, s, $D_2O$ exchangeable). This information confirms the presence of two hydroxyl groups in arthrosporone. One hydroxyl group in arthrosporone [**1**] forms part of a $\beta$-hydroxyketone functionality because **1** is readily dehydrated under acid catalysis (*p*-TsOH, $C_6H_6$, 78°, 3 h) to anhydroarthrosporone [**2**], an $\alpha,\beta$-unsaturated ketone.

Anhydroarthrosporone [**2**], $C_{15}H_{22}O_2$, has a uv spectrum which shows an unsaturated carbonyl chromophore [$\lambda_{max}$ 230 ($\epsilon$ = 13 700)], while its ir spectrum shows absorption bands characteristic of a hydroxyl (3462 $cm^{-1}$), an $\alpha,\beta$-unsaturated ketone (1693 and 1632 $cm^{-1}$), and a *gem*-dimethyl group (1372, 1364 $cm^{-1}$). The $^{13}C$-nmr spectrum of anhydroarthrosporone [**2**] displays three lowfield signals for $sp^2$-hybridized carbons at $\delta$ 211.6 (weak singlet, carbonyl carbon), $\delta$ 177.0 and $\delta$ 122.9 [singlet and doublet; disubstituted and monosubstituted olefinic carbons, respectively (12)]. A doublet at $\delta$ 63.4 suggests the presence of a methine carbon $\alpha$ to a carbonyl (13). These data suggest that anhydroarthrosporone contains a $\beta$-substituted $\alpha,\beta$-unsaturated cyclopentenone. The $^{13}C$-nmr spectrum also shows the presence of a quaternary carbon bearing an hydroxyl group ($\delta$ 92.7) (14,15), a quaternary carbon, two CH, three $CH_2$, and four Me groups.

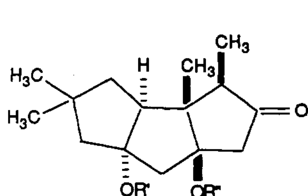
The $^{1}H$-nmr spectrum of anhydroarthrosporone [**2**] indicates a vinyl hydrogen ($\delta$ 5.85) coupled to a methylene hydrogen ($\delta$ 2.72), methyl hydrogens ($\delta$ 1.11, d) coupled to a vicinal methine hydrogen ($\delta$ 2.34, $J$ = 7 Hz), three quaternary methyl groups [$\delta$ 1.20 (s), 1.13 (s), 0.94 (s)] and a series of one-hydrogen multiplets (Table 1). The hrms of anhydroarthrosporone [**2**] exhibits fragment ions at *m/z* 216 $[M-H_2O]^+$, 123 $[M-C_7H_{11}O]^+$, and base peak at *m/z* 122 ($C_8H_{10}O$).

All the spectroscopic data presented for anhydroarthrosporone and its acetyl derivative, *O*-acetylanhydroarthrosporne [**7**], are consistent with either a hirsutane skeleton [**2**] or with skeleton **8**. The $^{1}H$-nmr spectrum of *O*-acetylanhydroarthrosporone [**7**] (Table 1), when compared with that of anhydroarthrosporone, shows deshielding of a methine hydrogen, two methylene hydrogens, and one of the quaternary methyl hydrogens. For either structure **2** or **8** the deshielded hydrogens would be those vicinal and cis to the acetoxyl group (16). The carbon skeleton of the arthroconidial metabolites was deduced in the following way. Compounds such as **2** or **8** would be expected to undergo a retro-aldol cleavage to give bicyclic compounds **9** or **10**, respectively. Compound **9** may be distinguished from **10** by its ir spectrum [cyclobutanone carbonyl absorption near 1780 $cm^{-1}$, cyclopentanone carbonyl absorption around 1745 $cm^{-1}$ (17,18)]. Accordingly, treatment of anhydroarthrosporone with excess NaH in dry $C_6H_6$ gave a single product in quantitative yield. The product is less polar than the starting metabolite; it is optically active and has a molecular formula of $C_{15}H_{22}O_2$ (hrms), as expected. Its uv spectrum shows a maximum characteristic of an $\beta$-alkyl sub-

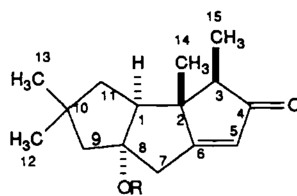
stituted cyclopentenone [$\lambda_{max}$ 223 nm (observed), 226 nm (calculated) (19)]. Its ir spectrum does not show hydroxyl absorption but has a strong carbonyl absorption band at 1738 $cm^{-1}$ indicative of the presence of a cyclopentanone as well as strong absorption bands due to an $\alpha,\beta$-unsaturated cyclopentenone. The hrms fragmentation pattern of the product shows a base peak at *m/z* 123 ($C_8H_{10}O + H$). These data suggest that the retro-aldol cleavage product has structure **9**. The $^1$H-nmr spectrum of **9** (see Experimental) gives further support to the assigned structure.

It follows that anhydroarthrosporone possesses the hirsutane skeleton **2** and that *O*-acetylanhydroarthrosporone is **7**. The assignment of the relative stereochemistry in the arthroconidial metabolites was based on the results of the following experiments. The cis, anti, cis configuration consistently found in the hirsutane series is maintained, and the angular methyl at C-2 is arbitrarily assigned a $\beta$ orientation. The assignment is supported by $^1$H-nmr pyridine shift studies (16, 17, 20) [$\Delta = \delta(CDCl_3) - \delta(C_5D_5N)$] carried out on compound **2**, which showed that the C-8 hydroxyl is cis to H-1 ($\Delta = -0.41$) and trans to the angular methyl group at C-2 ($\Delta = -0.08$). Because no conclusions could be drawn about the stereochemistry of the secondary methyl group at C-3, $^1$H-nmr pyridine-induced shift studies were also carried out on several derivatives of **2**.

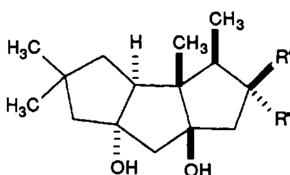
Reduction of *O*-acetylanhydroarthrosporone [**7**] with $NaBH_4/CeCl_3$ (21) in MeOH gave a mixture of the allylic alcohol **11** and the epimeric saturated acetoxyalcohols **12** and **13**. The composition of the reduction product was not affected by reaction temperature, order of addition of reagents, or omission of $CeCl_3$. The reduction of the C,C



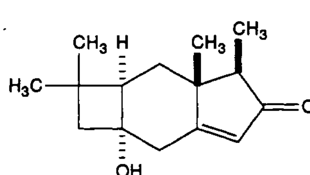

**1** R′=R″=H
**5** R′=Ac, R″=H
**6** R′=R″=Ac



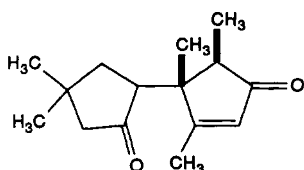

**2** R=H
**7** R=Ac



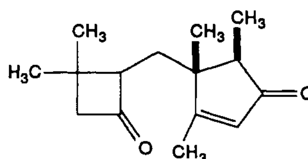

**3** R′=H, R″=OH
**4** R′=OH, R″=H
**16** R″=H, R″=OAc




**8**




**9**




**10**

double bond of *O*-acetylanhydroarthrosporone [**7**] is expected to take place from the less sterically hindered β face of the molecule because reduction of the structurally similar bicyclic enone **14** proceeds in this manner (22). A pyridine-induced shift study of **11** shows that H-3 is strongly deshielded (Δ = −0.46) whereas the methyl group at C-2 shows no appreciable shift (Table 1) on solvent change. These observations suggest that

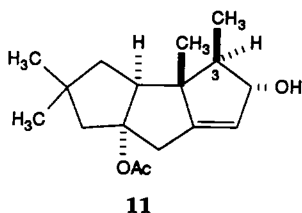
TABLE 1. [1]H-nmr Data[a] and Solvent Studies for Compounds **1**, **2**, **5**–**7**, **11**–**13**.

| Proton | Solvent | Compound | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | **1** | **2** | **5** | **6** | **7** | **11** | **12** | **13** |
| H-1 | CDCl$_3$ | 2.56 dd (8,12) | 2.38 dd (9,11) | 2.83 dd (9,12) | 2.86 dd (8,12) | 2.68 t (10) | 2.57 dd (9,10) | 2.67 dd (8,13) | 2.67 dd (8,12.5) |
| | pyridine-$d_5$ | 2.77 dd (9.5,12) | 2.63 t (10) | 2.97 dd (8,12) | | | 2.70 t (10) | 2.85 dd (8,12) | 2.84 dd (8,12.5) |
| H-3 | CDCl$_3$ | 2.57 q (7) | 2.32 q (7) | 2.34 dq (1,7) | 2.27 dq (1,7) | 2.33 q (7.5) | 1.81 dq (6,7) | 1.97 dq (7,9) | 2.35 m |
| | pyridine-$d_5$ | 2.92 dq (1,7) | 2.41 q (7.5) | 2.52 q | | | 2.26 dq (6,7) | 2.38 dq (7,8) | 2.34 dq (4,8) |
| H-4 | CDCl$_3$ | | | | | | 4.56 m | 3.89 dt (6,9) | 4.31 dd (3,6) |
| | pyridine-$d_5$ | | | | | | 4.94 m | 4.18 m | 4.52 m |
| H-5 | CDCl$_3$ | 2.69 dd (1,20) | 5.85 d (1.3) | 2.64 dd (1,19) | 3.21 dd (1,19) | 5.75 d (2) | 5.29 br s | 2.08 m | 1.96 ddd (4,6,14) |
| | | 2.20 d (20) | | 2.22 d (19) | 2.29 d (19) | | | 1.38 ddd (4,6,13) | 1.88 m |
| | pyridine-$d_5$ | 2.55 d (20) | 5.92 d (2) | 2.69 d (19) | | | 5.66 d (2) | 2.04 ddd (4,8,11) | 2.13 ddd (3,8,14) |
| | | 2.47 dd (1,20) | | 2.44 d (19) | | | | 1.75 ddd (4,6,15) | 2.01 ddd (4,6,14) |
| H-6 | CDCl$_3$ | | | | | | | 2.29 m | 2.35 m |
| | pyridine-$d_5$ | | | | | | | 2.40 m | |
| H-7 | CDCl$_3$ | 2.40 d (16) | 2.79 d (16) | 2.81 d (16) | 3.31 d (17) | 3.30 d (17) | 3.02 dd (1,17) | 2.43 dd (11,18) | 2.37 dd (9,10) |
| | | 2.21 d (16) | 2.72 dd (1,16) | 2.24 d (15.5) | 2.18 d (17) | 2.74 d (2,17) | 2.33 (2,17) | 2.08 dd (8,18) | |
| | pyridine-$d_5$ | 2.75 d (16) | 2.96 d (15) | 3.10 d (15.5) | | | 3.29 d (16) | 2.59 dd (9,15) | 2.46 dd (9,15) |
| | | 2.46 d (16) | 2.80 dd (2,15) | 2.45 d (16) | | | 2.46 dd (2,16) | 2.46 dd (4,8) | 2.11 dd (7,15) |
| H-9 | CDCl$_3$ | 1.96 d (14) | 1.87 dd (2,14) | 2.28 dd (3,15) | 2.31 d (15) | 2.43 d (15) | 2.29 dq (2,15) | 2.11 dd (3,15) | 1.80 d (15) |
| | | 1.79 dd (2.5,14) | 1.68 d (14) | 1.86 d (15) | 1.59 d (15) | 1.64 d (15) | 1.53 d (15) | 1.75 d (15) | 1.85 d (15) |
| | pyridine-$d_5$ | 2.14 d (14) | 2.08 dd (2,14) | 2.46 d (15) | | | 2.52 dd (2,15) | 2.29 dd (3,15) | 2.27 dd (3,15) |
| | | 2.06 dd (3,14) | 1.68 d (14) | 2.15 d (15) | | | 1.62 d (15) | 1.83 d (15) | 1.85 d (15) |
| H-11 | CDCl$_3$ | 1.70 t (12) | 1.72 ddd (2,9,13) | 1.69 t (12) | 1.67 t (12) | 1.69 ddd (2,9,12.5) | 1.52 ddd (2,9,13) | 1.48 ddd (3,8,13) | 1.30 t (13) |
| | | 1.59 ddd (2.5,8,12) | 1.47 dd (11,13) | 1.56 ddd (3,9,12) | 1.62 dd (8,12) | 1.45 dd (9,12.5) | 1.32 dd (10,13) | 1.22 t (13) | 1.53 ddd (3,8,13) |
| | pyridine-$d_5$ | 1.97 t (12) | 1.65 ddd (2,9,13) | 2.00 t (12) | | | 1.46 ddd (2,9,13) | 1.47 ddd (3,8,13) | 1.49 ddd (3,8,13) |
| | | 1.47 ddd (3,9.5,12) | 1.45 dd (10,13) | 1.58 ddd (3,8,12) | | | 1.33 dd (11,13) | 1.25 t (12) | 1.31 t |
| H-12 | CDCl$_3$ | 1.16 s | 1.20 s | 1.04 s | 1.03 s | 0.93 s | 1.03 s | 1.05 s | 1.02 s |
| | pyridine-$d_5$ | 1.24 s | 1.40 s | 1.01 s | | | 1.06 s | 1.09 s | 1.08 s |
| H-13 | CDCl$_3$ | 1.08 s | 1.13 s | 0.89 s | 1.06 s | 1.11 s | 0.80 s | 1.05 s | 1.04 s |
| | pyridine-$d_5$ | 0.96 s | 1.10 s | 1.07 s | | | 0.83 s | 1.03 s | 1.02 s |
| H-14 | CDCl$_3$ | 0.84 s | 0.94 s | 1.08 s | 0.88 s | 1.09 s | 1.05 s | 0.80 s | 0.95 s |
| | pyridine-$d_5$ | 0.94 s | 0.86 s | 1.09 s | | | 1.02 s | 0.83 s | 1.21 s |
| H-15 | CDCl$_3$ | 1.02 d (7) | 1.11 d (7) | 1.06 d (7) | 1.05 d (7) | 1.10 d (7.5) | 1.09 d (7.5) | 1.03 d (7) | 1.00 d (7) |
| | pyridine-$d_5$ | 1.00 d (7) | 1.14 d (7.5) | 1.11 d (7) | | | 1.26 d (7.5) | 1.22 d (7) | 1.25 d (8) |
| COC*H*$_3$ | CDCl$_3$ | | | 1.98 s | 1.98 s<br>2.04 s | 1.98 s | 1.97 s | 2.00 s | 2.02 s |
| | pyridine-$d_5$ | | | 1.95 s | | | 1.93 s | 1.88 s | 2.01 s |
| OH | CDCl$_3$ | | 1.63 s | 1.47 s | | | | | 2.23 s |
| | pyridine-$d_5$ | 5.60 s | 6.21 s | | | | 6.34 d (4) | 6.26 d (4) | 5.82 d (2) |

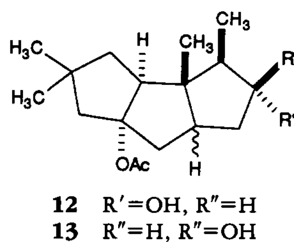
[a]Reported as chemical shift (δ), multiplicity, and coupling constant in Hz (in parentheses).

the hydroxyl at C-4 is cis to H-3 and trans to the C-2 methyl. Thus, in allylic alcohol **11** the C-3 methyl is β while the hydroxyl group is α.

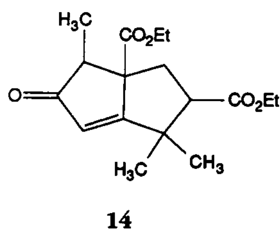
The ir spectra of both acetoxyalcohols **12** and **13** show absorption bands characteristic of hydroxyl and acetoxyl functional groups, while the $^{1}H$-nmr spectra of **12** and **13** each show a carbinyl hydrogen, a methyl doublet, an acetoxyl methyl, and three methyl singlets. A pyridine-induced shift study of acetoxyalcohol **12**, which was the major isomer, shows that the C-4 hydroxyl is α oriented since it is cis to H-3 ($\Delta = -0.41$) and trans to the angular methyl at C-2 ($\Delta = -0.03$). On the other hand, the pyridine-induced shift study of the minor epimer, compound **13**, shows that the C-4 hydroxyl is β oriented, because the angular methyl at C-2 and the secondary methyl at C-3 each undergo appreciable deshielding when the solvent is changed from $CDCl_3$ to $C_5D_5N$ whereas the chemical shift of H-3 remains unchanged. Correlation experiments were undertaken to confirm the structure of compound **12**. Catalytic hydrogenation of anhydroarthrosporone gave a single ketone, **15**, which was subjected to further reduction with $NaBH_4$. The epimeric mixture of diols obtained from the reduction of compound **15** was separated, and the spectral properties of the major product were identical in all respects with those of the diol obtained by deacetylation of acetoxyalcohol **12** with methanolic $Na_2CO_3$. It is interesting to note that pyridine shift studies of compounds **11** and **12** show that in each compound the secondary methyl at C-3 undergoes substantial downfield shifts ($\Delta = -0.17$ and $-0.19$, respectively). This may be explained on the basis of conformational considerations. Ring C of the triquinane system may adopt an envelope ($C_3$) conformation with C-3 being the flap of the envelope. Examination of molecular models reveals that in the $C_3$ conformation the C-4 hydroxyl group lies between the hydrogen and the methyl group at C-3.



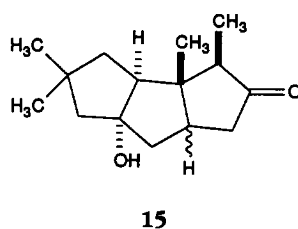

**11**




**12** R′=OH, R″=H
**13** R″=H, R″=OH




**14**




**15**

The stereochemistry of arthrosporone [**1**] was assigned as follows. The orientation of the C-3 methyl of arthrosporone should be the same as that in anhydroarthrosporone [**2**], provided that no isomerization took place during the dehydration reaction. Evidence that the stereochemical integrity is retained is provided by $^{1}H$-nmr data: H-3 resonates at δ 2.57 in arthrosporone [**1**] and at δ 2.54 in ketone **15**, indicating a similar environment for this hydrogen in each compound. Comparison of the $^{1}H$-nmr spectra of **1** and *O*-acetylarthrosporone [**5**] reveals that H-3 shifts upfield ($-0.22$) upon acetylation of the C-8 hydroxyl, indicating that H-3 and the C-8 hydroxyl are proximate to one another. Results of $^{1}H$-nmr pyridine-induced shift studies of compounds **1** and **5** (Table 1) lend support to the stereochemical assignment at C-3 and C-6. In arthro-

sporone, H-3 is strongly deshielded ($\Delta = -0.35$) and the C-2 methyl (vicinal and cis to the C-6 hydroxyl) shows moderate deshielding ($\Delta = -0.10$), while the C-3 methyl is not shifted when the $^1$H-nmr spectrum recorded in $C_5D_5N$ is compared with that recorded in $CDCl_3$. Similar solvent shifts are observed for *O*-acetylarthrosporone [**5**]. Finally, nOe studies (23,24) of compound **1** show a 4% enhancement of the C-2 methyl upon irradiation of the C-3 methyl hydrogens. This data confirms that the C-3 methyl is β oriented in arthrosporone and, it follows, in anhydroarthrosporone.

The stereochemistry of arthrosporol is assigned as depicted in structure **3**. Oxidation of **3** with pyridinium chlorochromate gave arthrosporone [**1**] as the only product. This result indicates that the secondary methyl at C-3 has the same orientation in each of compounds **1** and **3**. The α orientation of the C-4 hydroxyl and β orientation of the C-6 hydroxyl were established by analysis of the acetoxyl-induced anisotropic shifts of the hydrogens vicinal to the hydroxyl groups of **3** and analysis of its mono-, di-, and triacetyl derivatives. Each derivative was prepared selectively as follows. 4-*O*-Acetylarthrosporol [**16**] was prepared by treatment of **3** with $Ac_2O$ in pyridine for 24 h or by treatment with $Ac_2O$, a catalytic amount of dimethylaminopyridine, and triethylamine for 3 h. The latter reaction, when allowed to proceed for 3 days, gave a quantitative yield of 4,8-*O,O*-diacetylarthrosporol [**17**]. 4,6,8,*O,O,O*-Triacetylarthrosporol [**18**] was obtained quantitatively when **3** was allowed to stir with a catalytic amount of *p*-toluenesulfonic acid in $Ac_2O$ for 3 h. Table 2 shows the $^1$H-nmr data for **3** and its acetyl derivatives. When there is an acetoxyl group at C-4 (e.g., **16**, **17**, **18**), a strong deshielding of H-3 and H-5 is observed. Further substitution of the C-8 hydroxyl with an acetoxyl group results in a downfield shift of H-1, one of H-7, and one of H-9, while H-3, which is very close in space to the C-8 acetoxyl, is shielded. When all three hydroxyl hydrogens are substituted with acetyl groups, both hydrogens at C-5 are deshielded, as would be expected for a trans relationship between the C-4 and C-6 hydroxyl groups. These data confirm the stereochemistry of arthrosporol to be that shown in **3**. Interestingly, 4-*O*-acetylarthrosporol [**16**] was isolated as a minor metabolite from the culture broth of the arthroconidial fungus.

Two minor metabolites were isolated from the culture broth. The less polar of these, which we have named dehydroarthrosporodione, has a molecular formula of $C_{15}H_{20}O_3$. It is optically active and contains a secondary hydroxyl (ir 3450 $cm^{-1}$; $^{13}$C nmr 75.1 d), a cyclopentanone (ir 1730 $cm^{-1}$; $^{13}$C nmr 218.8 s), a cyclopentenone (ir 1700; 1637 $cm^{-1}$; $^{13}$C nmr 205.8 s, 185.0 s, 145.0 s) and four methyl groups ($^1$H and $^{13}$C nmr). Its uv spectrum shows a maxima at 242 nm (α,β-substituted cyclopentenone), which increases in intensity but does not shift upon the addition of acid or base; this suggests that the hydroxyl group is not part of the chromophore (17,18). The $^1$H-nmr spectrum of dehydroarthrosporodione shows that the carbinyl hydrogen is allylic (δ 4.5, br s) with a small coupling to allylic methylene hydrogens [δ 2.83 (2.2 Hz), 2.21 (2 Hz)]. Decoupling experiments reveal that the allylic methylene hydrogens are also coupled to a methine hydrogen (δ 2.93, m) which is further coupled to two methylene hydrogens [δ 2.60 (4, 19 Hz), 2.39 (2, 9, 19 Hz)]. The size of the geminal coupling observed for the latter methylene indicates that it is α to a carbonyl. These data are consistent with the assignment of structure **19** for dehydroarthrosporodione but are also not inconsistent with the structure in which the carbonyl at C-11 and the hydroxyl at C-9 are interchanged.

Compound **19** readily forms the acetyl derivative **20** and when oxidized with pyridinium chlorochromate gives triketone **21**. The spectral properties of compounds **20** and **21** provide support for the substitution pattern in **19**. A downfield shift ($\Delta =$ 0.27, 0.30) is observed for the allylic methylene hydrogens when the $^1$H nmr of **21** is compared with the $^1$H nmr of **19**. Lack of material precluded further experiments with

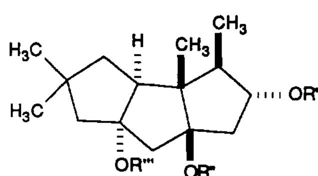
TABLE 2. [1]H-nmr Data[a] for Compounds **3**, **4**, **16–18**.

| Proton | Compound | | | | |
|---|---|---|---|---|---|
| | **3** | **4** | **16** | **17** | **18** |
| H-1 . . . . . . . . . | 2.40 dd (8,12) | 2.51 dd (5,14) | 2.41 dd (8,11) | 2.70 dd (9,12) | 2.75 dd (9,12) |
| H-3 . . . . . . . . . | 2.01 m | 1.87 d (14) | 2.27 dq (7,9) | 2.03 m | 2.02 m |
| H-4 . . . . . . . . . | 3.94 dt (5,9) | 4.14 dt (2,5) | 4.84 dt (4,9) | 4.82 ddd (4,9,10) | 4.85 dt (4,10) |
| H-5 . . . . . . . . . | 2.01 m | 2.07 d (5,7) | 2.13 dd (9,14) | 2.13 dd (10,15) | 2.43 dd (4,15) |
| | | 1.98 m | 1.96 d (4,14) | 1.88 dd (4,15) | 2.30 dd (10,15) |
| H-7 . . . . . . . . . | 2.32 d (15) | 2.31 dt (1.5,11) | 2.27 d (14) | 2.64 d (15) | 3.19 d (17) |
| | 2.22 d (15) | 2.13 dd (1.5,14) | 2.23 d (14) | 2.34 d (15) | 2.32 d (17) |
| H-9 . . . . . . . . . | 1.86 d (13) | 1.85 dd (2,14) | 1.91 d (14) | 2.20 dd (3,15) | 2.25 dd (3,15) |
| | 1.70 dd (2,13) | 1.48 m | 1.73 d (2.5,13) | 1.82 d (15) | 1.53 d (15) |
| H-11 . . . . . . . . . | 1.54 t (12) | 1.57 d (13.5) | 1.60 t (12) | 1.59 t (12) | 1.58 t (12) |
| | 1.47 ddd (2,8,12) | 1.48 m | 1.51 ddd (2.5,8,13) | 1.44 dd (3,8,12) | 1.49 ddd (3,9,12) |
| H-12 . . . . . . . . . | 1.11 s | 1.09 s | 1.09 s | 1.03 s | 1.01 s |
| H-13 . . . . . . . . . | 1.04 s | 1.03 s | 1.04 s | 1.01 s | 1.00 s |
| H-14 . . . . . . . . . | 0.76 s | 0.92 s | 0.78 s | 0.81 s | 0.84 s |
| H-15 . . . . . . . . . | 1.02 d (7) | 1.00 d (7) | 0.96 d (7) | 1.00 d (6.5) | 0.99 d (7) |
| COMe . . . . . . . . | | | 2.03 s | 2.04 s<br>2.02 s | 2.06 s<br>2.03 s<br>2.00 s |
| OH . . . . . . . . . | 1.77 s<br>1.64 s | 2.07 s<br>1.57 s | 1.63 s | | |

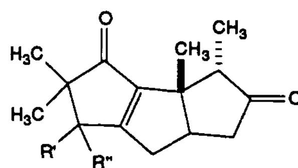
[a]Reported as chemical shift (δ), multiplicity, coupling constant in Hz (in parentheses); solvent $CDCl_3$.

this metabolite, and no unambiguous conclusions could be made about the stereochemistry of the C-3 methyl in **19**.

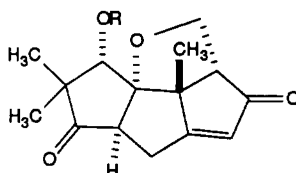
We tentatively assign structure **22** to the other minor metabolite. This compound has the molecular formula $C_{15}H_{18}O_4$ and is optically active. Its ir spectrum shows the presence of hydroxyl (3456 $cm^{-1}$), cyclopentanone carbonyl (1742 $cm^{-1}$), and a cyclopentenone α,β-unsaturated carbonyl (1704, 1629 $cm^{-1}$). Because compound **22** forms monoacetyl derivative **23** upon acetylation, the fourth oxygen of the molecular formula of **22** must be present as an ether. The $^1H$ nmr spectrum of **22** shows three methyl singlets. There is a vinyl hydrogen (δ 5.90, d) that shows allylic coupling to a methylene hydrogen (δ 2.76), which is further coupled to two other hydrogens (δ 3.33, 3.07, AB of ABX). Decoupling experiments show the presence of a second ABX spin system (δ 4.30, 3.98, 2.84). These data are consistent with structure **22**. Further evidence for the tentative structural assignment is based on the spectral properties of its acetyl derivative **23**. In the $^1H$-nmr spectrum of **23**, acetoxyl-induced anisotropic shifts are observed for the carbinyl hydrogen (1.17 ppm), H-8 (0.17 ppm), the geminal




**17** R′=R‴=Ac, R″=H
**18** R′=R″=R‴=Ac




**19** R′=H, R″=OH
**20** R′=H, R″=OAc
**21** R′=R″=O




**22** R=H
**23** R=Ac

methyl hydrogens, and the methylene hydrogens at C-15. These observations are consistent with the structure proposed. Because only very small amounts of compound **22** were obtained, no further experiments were possible.

Qualitative antifungal bioassay of the arthroconidial metabolites **1**, **2**, and **3** against several *Ceratocystis* species (7) reveals that these metabolites display weak antifungal activity, insufficient to account for the antifungal activity observed in the crude neutral extract unless their effect is synergistic. Further studies are required to establish which metabolites are responsible for the total antifungal activity.

## EXPERIMENTAL

General experimental procedures.—Distilled $H_2O$ was used to prepare growth media and was redistilled using an all-glass distillation apparatus. Difco bacto potato dextrose broth (PDB) or potato dextrose agar (PDA) and yeast extract were utilized for growth media. Still culture fermentation was performed using 2.5-liter Fernback flasks. Celite 545 (American Chemicals Ltd) was used as a filter aid.

Reagent grade solvents were distilled prior to use. Skellysolve B refers to Skelly Oil Company petroleum ether, bp 62–70°. Analytical grade $Et_2O$ (ACS 288) from freshly opened cans was used without further purification. Anhydrous $MgSO_4$ or anhydrous $Na_2SO_4$ was used a drying agent.

Cc was carried out using Brinkman Si gel 60 (0.08 mm, 200 mesh). E. Merck Si gel 60 (0.04–0.063 mm, 230–400 mesh) was used for flash cc (25). Analytical tlc was carried out using Si gel G containing 1% Retman P-1 electronic phosphor (General Electric) or on aluminum-foil-supported Si gel 60 (E. Merck, 0.25 mm, $F_{254}$) plates. The chromatograms were examined under uv light (λ 254 or λ 350 nm). The developed plates were visualized by spraying with aqueous 20% $H_2SO_4$ containing 1% vanillin (reagent A) or by dipping in 5% phosphomolybdic acid in aqueous 5% $H_2SO_4$ containing a trace of ceric sulfate (reagent B), followed by careful charring on a hot plate. Cholesterol was used as a standard and $R'_f$ values are reported for the $R_f$ of each metabolite relative to the $R_f$ of cholesterol, which was assigned the value of 1.00 in the reported solvent system.

Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Ft-ir spectra were obtained on a Nicolet 7199 F.T. interferometer. Uv spectra were recorded on a Unicam SP 1700 spectrometer coupled to a 7470A plotter. Optical rotations were determined on a Perkin-Elmer model 141 automatic polarimeter. High resolution electron impact mass spectra (hreims) were recorded on an AEI MS-50 mass spectrometer coupled to a data processing DS-50 computer system. Cims and low resolution electron impact mass spectra (lreims) were determined on an AEI MS-4 mass spectrometer coupled to a DS-9 computer system. Data are reported as *m/z* (rel. int.). Only diagnostically significant peaks or those greater than 10% of the base peak are reported.

$^1$H-nmr and $^{13}$C-nmr spectra were recorded on a Bruker WH-400 spectrometer coupled to an Aspect 2000 computer system. Chemical shifts expressed in δ units are reported in ppm downfield from the inter-

nal standard TMS. Coupling constants, $J$, are expressed in Hz, and the following abbreviations are used: m = multiplet, s = singlet, d = doublet, t = triplet, q = quartet, br = broad.

CULTURING OF THE ARTHROCONIDIAL FUNGUS.—The strain of arthroconidial fungus used in these studies was isolated by Drs. Y. Hiratsuka and A. Tsuneda, Northern Forest Research Centre, Canadian Forestry Service, Edmonton, as a contaminant on a culture of *C. ulmi*. The fungus, believed to be a haploid basidiomycete, has not yet been fully identified. A culture was deposited at the University of Alberta Microfungus Collection under accession number UAMH 4262.

The culture was maintained at 4° in agar slant tubes containing PDA and 0.2% yeast extract (PDAY). Inoculum was prepared by adding 2 ml of sterile $H_2O$ to a slant culture and gently scratching the surface of the culture with a sterile needle to produce a mycelial suspension. The suspension was used to inoculate two PDAY plates. Culture growth occurred at 15 to 20° over 2 weeks; then a mycelial suspension, prepared as described above from the plate culture, was transferred to two 500-ml Erlenmeyer flasks containing 200 ml sterilized PDB containing 0.2% yeast extract (PDBY). This culture was maintained at 15 to 20° on a gyrotory shaker for 2 weeks. Still cultures (10 liters) were prepared by transferring 25 ml of inoculum into ten Fernbach flasks each containing 1 liter of sterile PDBY.

The fungus was harvested after growth for 1 month at 15 to 20°. The mycelium was separated from the broth by filtration through glass wool. The mycelial mat was dried in a fume hood for 10 days, pulverized, and continuously extracted in a Soxhlet extractor with $Et_2O$ followed by EtOAc. Each organic extract was washed, dried, and concentrated to give crude mycelial extracts ($Et_2O$ extract, 0.15 g/liter; EtOAc extract, 0.24 g/liter).

The broth was clarified by filtration through a filter aid, then concentrated to ⅓ volume at reduced pressure. The concentrate was continuously extracted for 2 days in a liquid-liquid extractor with $Et_2O$, then EtOAc. The organic extracts were dried and concentrated to give crude broth extracts ($Et_2O$ extract, 0.11 g/liter; EtOAc extract, 0.04 g/liter).

PRELIMINARY FRACTIONATION OF THE CRUDE EXTRACTS.—The $Et_2O$-soluble extracts (2.15 g) were dissolved in EtOAc and separated into neutral, strong acidic, and weak acidic compounds by standard acid-base extraction (neutral extract, 0.97 g; strong acid extract, 0.15 g; weak acid extract, 0.39 g).

SEPARATION AND ISOLATION OF THE NEUTRAL METABOLITES.—The neutral extract was separated by flash chromatography using $CH_2Cl_2$-MeCN (3:2). The fractions were analyzed by tlc and like fractions combined to give fractions A–E. Fraction A (83 mg) yielded **19** and **16**; fraction B (17 mg) **1**, **2**, and **19**; fraction C (147 mg) **1**, **2**, and **22**; fraction D (20 mg) **1**, **2**, and cyclohumuladiol; fraction E (50 mg) **3** and an unidentified metabolite. The individual components of each fraction were isolated by a combination of cc and preparative tlc. A complete description of the isolation of each of these sesquiterpenes, including the identification of cyclohumuladiol, may be found in Amouzou (7).

ARTHROSPORONE [**1**].—$R'_f$ 0.50 [$Me_2CO$-$C_6H_6$ (3:2)]; recrystallized from Skellysolve B/$Et_2O$ as colorless needles: mp 139–141°; $[\alpha]_D$ −140.8° ($c$ = 0.9, $CHCl_3$); λ max (MeOH) 280 nm (ε = 650); Ft-ir ($CHCl_3$ cast) 3440, 2951, 2866, 1731, 1381, 1360, 1275, 1189, 1015 $cm^{-1}$; $^{13}C$ nmr ($CDCl_3$) 216.4, (s, C-4), 91.0 (s, C-6), 87.0 (s, C-8), 60.6 (d, C-3), 58.9 (t, C-5), 56.9 (t, C-7), 55.5 (d, C-1), 54.6 (s, C-2), 49.9 (t, C-9), 44.7 (t, C-11), 40.2 (s, C-10), 29.6 (q), 26.9 (q), 11.3 (q), 8.3 (q); cims ($NH_3$) $m/z$ $[M + NH_4]^+$ 270 (100), $[M]^+$ 252 (40); hreims (252.1722 calcd for $C_{15}H_{24}O_3$) found 252.1723 (36), 235 (38), 234 (28), 219 (13), 216 (2), 206 (12), 192 (89), 191 (24), 177 (22), 163 (21), 125 (100), 102 (25), 95 (33), 83 (40).

ANHYDROARTHROSPORONE [**2**].—$R'_f$ 0.74 [$Me_2CO$-$C_6H_6$ (2:3)]; recrystallized from Skellysolve B/$Et_2O$: mp 118–119°; $[\alpha]_D$ +62° ($c$ = 2.0, $CHCl_3$); λ max (MeOH) 230 nm (ε = 13,700); Ft-ir ($CHCl_3$ cast) 3461, 1693, 1632, 1466, 1448, 1372, 1364, 872 $cm^{-1}$; $^{13}C$ nmr ($CDCl_3$) 211.6 (s, C-4), 177.0 (s, C-6), 122.9 (d, C-5), 92.7 (s, C-8), 63.4 (d, C-3), 57.7 (d, C-1), 55.9 (t, C-7), 53.4 (s, C-2), 44.0 (t, C-9), 43.3 (s, C-10), 41.8 (t, C-11), 30.2 (q), 28.2 (q), 21.8 (q, C-14), 9.5 (q, C-15); cims ($NH_3$) $m/z$ $[M + NH_4]^+$ 252 (2), $[M + H]^+$ 235 (100), 234 (20); hreims (234.1616 calcd for $C_{15}H_{22}O_2$) found 234.1617 (63), 219 (32), 216 (34), 201 (23), 173 (23), 123 (78), 122 (100), 111 (10), 95 (13), 55 (20).

ARTHROSPOROL [**3**].—$R'_f$ 0.39 [$Me_2CO$-$C_6H_6$ (3:2)]; recrystallized from $Et_2O$: mp 163–164°; $[\alpha]_D$ −29° ($c$ = 2.0, $CHCl_3$), −62.1 ($c$ = 1.0, MeOH); Ft-ir ($CHCl_3$ cast) 3376, 2951, 2933, 2456, 1380, 1372, 1088, 1012 $cm^{-1}$; $^{13}C$ nmr ($CDCl_3$) 91.0 (s, C-6 or -8), 90.3 (s, C-8 or -6), 76.8 (d, C-4), 60.9 (d, C-3 or -1), 58.5 (t, C-5 or -7), 58.2 (t, C-7 or -5), 56.3 (s, C-2), 52.1 (d, C-1 or -3), 49.0 (t, C-11 or -9), 45.3 (t, C-9 or -11), 40.0 (s, C-10), 30.7 (q), 28.6 (q), 12.9 (q), 12.5 (q); cims ($NH_3$) $m/z$ $[M + NH_4]^+$ 272 (100), 254 (18), 237 (15); hreims (236.1776 calcd for $C_{15}H_{24}O_2$) found $[M - H_2O]^+$ 236.1778 (11), 218 (100), 203 (31), 190 (8), 182 (38), 174 (33), 150 (54), 139 (32), 135 (33), 127 (32), 125 (16), 123 (117), 121 (15), 110 (25), 109 (38), 107 (34), 95 (31), 83 (54), 69 (38), 55 (65).

4-*O*-ACETYLARTHROSPOROL [**16**].—$R'_f$ 0.70 [$Me_2CO$-$C_6H_6$ (2:7)]; recrystallized from Skelly-solve B/$Et_2O$: mp 132–135°; [α]D −60.9° (*c* = 1.4, $CHCl_3$); Ft-ir ($CHCl_3$ cast) 3450, 3440, 1720, 1261, 1021 $cm^{-1}$; cims ($NH_3$) *m/z* $[M+NH_4]^+$ 314 (100), 296 (18), 236 (2); hreims (278.1883 calcd for $C_{17}H_{26}O_3$) found 278.1875 (0.2), 263 (0.2), 236 (3.5), 218 (100), 203 (18), 200 (0.8) 160 (14), 139 (13), 136 (41), 109 (25), 55 (23).

ARTHROSPORODIONE [**19**].—$R'_f$ 0.74 [$Me_2CO$-$C_6H_6$ (2:7)]; [α]D −80.1° (*c* = 2.5, $CHCl_3$); λ max (MeOH) 242 nm (ε = 7712), λ max (MeOH + 0.1 N HCl) 242 nm (ε = 8142), λ max (MeOH + 0.1 N NaOH) 242 nm (ε = 10,594); Ft-ir ($CHCl_3$ cast) 3450, 1738, 1700, 1688, 1637, 1374, 1089 $cm^{-1}$; $^{13}C$ nmr ($CDCl_3$) 218.8 (s, C-4), 205.8 (s, C-11), 185.0 (s, C-8), 145.1 (s, C-1), 75.1 (d, C-9), 54.3 (s, C-10), 51.0 (d, C-3 or -6), 49.9 (d, C-6 or -3), 42.1 (s, C-2), 41.7 (t, C-5 or -7), 31.7 (t, C-7 or -5), 23.6 (q), 20.2 (q), 18.5 (q), 9.3 (q); cims ($NH_3$) *m/z* $[M+NH_4]^+$ 266 (100), 248 (18), 235 (14); hreims (248.1412 calcd for $C_{15}H_{20}O_3$) found 248.1417 (42), 233 (100), 230 (4), 215 (5), 192 (11), 177 (19), 173 (7), 131 (13), 91 (17).

COMPOUND **22**.—$R'_f$ 0.23 [EtOAc-pentane (2:3)]; [α]D +28.9° (*c* = 0.92, $CHCl_3$); λ max (MeOH) 229 nm (ε = 19,400); Ft-ir ($CHCl_3$ cast) 3456, 3070, 1740, 1704, 1629, 1080 $cm^{-1}$; cims ($NH_3$) *m/z* $[M+NH_4]^+$ 280 (100), 263 (43), 262 (21); hreims (262.1205 calcd for $C_{15}H_{18}O_4$) found 262.1207 (100), 234 (3), 191 (37), 163 (12), 72 (2); $^1H$ nmr ($CDCl_3$-$D_2O$) δ 5.90 (1H, d, 2.0 Hz, H-5), 4.29 (1H, t, 9.6 Hz, H-15α), 3.98 (1H, dd, 9.0, 15.5 Hz, H-15β), 3.92 (1H, s, H-11), 3.33 (1H, t, 9.0 Hz, H-8), 3.07 (1H, dd, 9.0, 15.5 Hz, H-7α), 2.84 (1H, dd, 3.0, 9.5 Hz, H-3), 2.76 (1H, ddd, 2.2, 9.0, 16.0 Hz, H-7β), 1.25 (3H, s, 10-Me), 1.15 (6H, s, 10-Me, 2-Me).

8-*O*-ACETYLARTHROSPORONE [**5**].—Arthrosporone [**1**] (2.4 mg) was dissolved in $NEt_3$ (2 ml), and *N,N*-dimethylaminopyridine (DMAP, 1.5 mg) and $Ac_2O$ (4 drops) were added. The mixture was stirred at room temperature for 3 days, then concentrated. The residue was partitioned between EtOAc (20 ml) and 5% aqueous HCl (5 ml). The organic extract was washed with $H_2O$, dried and concentrated. Purification by flash chromatography gave an analytically pure sample of compound **5**: mp 120–122°; $R_f$ 0.58 [EtOAc-pentane (1:2)]; [α]D −37.6° (*c* = 5.0, $CHCl_3$); Ft-ir ($CHCl_3$ cast) 3449, 1736, 1382, 1368, 1249, 1017 $cm^{-1}$; hreims (294.1824 calcd for $C_{17}H_{24}O_3$) found 294.1818 (1), 252 (1.3), 234 (32), 216 (3), 192 (100), 125 (92), 110 (31).

6,8-*O,O*-DIACETYLARTHROSPORONE [**6**].—Arthrosporone [**1**] (2 mg) and $Ac_2O$ (5 drops) were mixed in $CH_2Cl_2$ (2 ml). The solution was cooled in an ice bath, and *p*-toluenesulfonic acid (few grains) was added. The mixture was allowed to warm to room temperature over a period of 60 min. The mixture was diluted with $CH_2Cl_2$ (10 ml), washed with aqueous 5% $NaHCO_3$ (2 × 2 ml) and brine (2 ml), dried, and concentrated. Purification of the residue by chromatography gave diacetate **6** as a waxy solid: $R'_f$ 1.18 [EtOAc-pentane (1:3)] Ft-ir ($CHCl_3$ cast) 1739, 1383, 1369, 1249, 1227, 1029 $cm^{-1}$.

8-*O*-ACETYLANHYDROARTHROSPORONE [**7**].—Anhydroarthrosporone [**2**] (6 mg), DMAP (catalytic amount), $Ac_2O$ (5 drops), and $NEt_3$ (2 ml) was allowed to stand at room temperature for 7 h. The solution was concentrated and the residue was partitioned between $CH_2Cl_2$ (20 ml) and 5% aqueous HCl (2 × 2 ml). The organic phase was dried, concentrated, and purified by chromatography to give compound **7** as a colorless liquid which slowly solidified over time: mp 76–77°; $R'_f$ 1.00 [EtOAc-pentane (1:2)]; [α]D +78.0° (*c* = 6.4, $CHCl_3$); λ max (MeOH) 228 nm (ε = 8273); Ft-ir ($CHCl_3$ cast) 1728, 1706, 1638, 1241 $cm^{-1}$; hreims (276.1719 calcd for $C_{17}H_{24}O_3$) found 276.1724 (2), 234 (4), 216 (100), 201 (39), 173 (36), 122 (27).

REDUCTION OF **1**.—Arthrosporone [**1**] (5 mg) was dissolved in MeOH (2 ml), and $NaBH_4$ (7 mg) was added. The mixture was stirred at room temperature for 1 h. Aqueous HOAc (50%, 1 ml) was added and the mixture stirred for 2 min. MeOH was removed at reduced pressure, $H_2O$ (2 ml) added, and the aqueous solution extracted with EtOAc. The organic extract was washed with brine, dried, concentrated, and purified by chromatography. Two products were obtained: arthrosporol [**3**] and epiarthrosporol [**4**]. Compound **4**: mp 150°, $R'_f$ 0.63 [$C_6H_6$-$Me_2CO$ (3:2)]; [α]D −64.0° (*c* = 0.1, MeOH); Ft-ir ($CHCl_3$ cast) 3301, 2950, 2930, 1378, 1364, 1067, 1024 $cm^{-1}$; hreims (254.1875 calcd for $C_{15}H_{26}O_3$) found 254.1875 (3), 236 (20), 218 (8), 182 (100), 177 (4), 174 (21), 164 (7), 150 (39), 139 (42), 127 (18), 124 (4), 121 (22), 111 (5), 83 (14), 55 (27).

DEHYDRATION OF **1** TO **2**.—Arthrosporone [**1**] (2 mg) was dissolved in dry $C_6H_6$ (3 ml) containing molecular sieves (3Å) and *p*-toluenesulfonic acid (catalytic amount). The mixture was stirred at room temperature for 4 h. The solution was decanted and diluted with $Et_2O$. The organic solution was washed with 10% $NaHCO_3$ (2 × 2 ml), dried, concentrated, and purified by chromatography to give anhydroarthrosporone [**2**], identical in all respects with the natural metabolite.

CONVERSION OF **2** TO BICYCLOKETONE **9**.—A solution of anhydroarthrosporone [**2**] (4.1 mg) in dry $C_6H_6$ (2 ml) was flushed with $N_2$ for 10 min. NaH (excess) was added as a suspension in $C_6H_6$ to the solution. The mixture was stirred at room temperature under an $N_2$ atmosphere for 5 h. The mixture was diluted with $C_6H_6$, quenched with 10% HOAc, then stirred until the excess NaH dissolved. The organic layer was separated, washed with brine, dried, and concentrated. The crude residue was purified by chromatography to give compound **9** in quantitative yield: mp 55–56°; $R'_f$ 1.29 [$C_6H_6$-$Me_2CO$ (8:1)]; $[\alpha]_D$ $-202°$ ($c$ = 1.95, $CHCl_3$); $\lambda$ max (MeOH) 223 nm ($\epsilon$ = 13,400); Ft-ir ($CHCl_3$ cast) 1738, 1702, 1625 $cm^{-1}$; $^1H$ nmr ($CDCl_3$) $\delta$ 5.92 (1H, q, 1.6 Hz, H-5), 2.58 (1H, ddd, 12.5, 8.5, 1.5 Hz, H-1), 2.20 (1H, ddd, 18.0, 2.4, 1.5 Hz, H-9$\alpha$), 2.08 (1H, q, 7.5 Hz, H-3), 2.05 (3H, d, 1.6 Hz, H-7), 2.04 (1H, dd, 18.0, 1 Hz, H-9$\beta$), 1.67 (1H, ddd, 13.0, 8.5, 2.5 Hz, HO-11$\alpha$), 1.45 (3H, s, H-14), 1.35 (1H, br t, 12.5 Hz, H-11$\beta$), 1.19 (3H, s, H-13), 1.08 (3H, d, 7.5 Hz, H-15), 1.04 (3H, s, H-12); hreims (234.1614 calcd for $C_{15}H_{22}O_2$) found 234.1623 (59), 219 (7), 150 (37), 124 (36), 123 (100), 122 (90), 112 (26), 97 (15), 95 (50).

OXIDATION OF ARTHROSPOROL [**3**].—Arthrosporol [**3**] (3 mg) in $CH_2Cl_2$ and pyridinium chlorochromate (PCC, 2.2 mg) was stirred at room temperature for 2 h. The suspension was filtered through a short column of florisil. The florisil column was washed with $Et_2O$, and the organic filtrates were combined and concentrated. Crystallization from Skellysolve B/$Et_2O$ gave arthrosporone [**1**], identical in all respects with the natural metabolite.

REDUCTION OF 8-*O*-ACETYLANHYDROARTHROSPORONE [**7**].—Compound **7** (5 mg) was dissolved in MeOH (1.5 ml). $CeCl_3$ (2.2 mg) and $NaBH_4$ (10 mg) were added and the mixture stirred at room temperature for 1 h. The mixture was worked up in the usual way and the crude reaction product purified by chromatography to give three products: compounds **11**, **12**, and **13**.

*Compound* **11**.—$R'_f$ 0.83 [$C_6H_6$-$Me_2CO$ (3:1)]; Ft-ir ($CHCl_3$ cast) 3400, 1732, 1676, 1242, 1017 $cm^{-1}$; cims ($NH_3$) $m/z$ $[M+NH_4]^+$ 296 (6), 278 (6), 201 (100); hreims (278.1875 calcd for $C_{17}H_{26}O_3$) found 278.1873 (1), 218 (100), 203 (35), 200 (4), 106 (85), 91 (20).

*Compound* **12**.—$R'_f$ 0.93 [$C_6H_6$-$Me_2CO$ (3:1)]; Ft-ir ($CHCl_3$ cast) 3464, 1732, 1724, 1367, 1245, 1016 $cm^{-1}$; cims ($NH_3$) $m/z$ $[M+NH_4]^+$ 298 (64); hreims 220 (32), 202 (5), 162 (22), 161 (56), 148 (65), 110 (100), 107 (26).

*Compound* **13**.—$R'_f$ 0.65 [$C_6H_6$-$Me_2CO$ (3:1)]; Ft-ir ($CHCl_3$ cast) 3400, 1732, 1713, 1383, 1366, 1243, 1087, 1016 $cm^{-1}$; hreims 238 (0.5), 220 (41), 202 (66), 187 (23), 176 (16), 161 (52), 148 (100), 111 (19), 108 (34), 93 (25).

CORRELATION OF ANHYDROARTHROSPRONE [**2**] WITH COMPOUND **11**.—A mixture of compound **2** (10 mg), 10% methanolic HOAc (4 drops), and activated Pd on charcoal (catalytic amount) in MeOH (2 ml) was stirred under an $H_2$ atmosphere for 30 min. The mixture was filtered through celite and the celite washed with MeOH. The combined filtrate was concentrated to yield ketone **15** in quantitative yield: mp 115–116°; $R'_f$ 0.82 [$C_6H_6$-$Me_2CO$ (7:2)]; Ft-ir ($CHCl_3$ cast) 3502, 1727 $cm^{-1}$; hreims (236.1772 calcd for $C_{15}H_{24}O_3$) found 236.1773 (16), 221 (9), 218 (20), 203 (9), 176 (71), 161 (33), 124 (39), 110 (100), 109 (65), 83 (5).

Compound **15** (8 mg) in MeOH (3 ml) and excess $NaBH_4$ was stirred for 1 h. HOAc (5 drops) was added and the mixture stirred for 5 min. Solvents were removed and the residue was partitioned between $H_2O$ (5 ml) and EtOAc (3 × 10 ml). The organic extracts were dried and concentrated. Purification by chromatography gave two diols: major mp 139–140°; $R'_f$ 0.14 [$C_6H_6$-$Me_2CO$ (3:2)]; Ft-ir ($CHCl_3$ cast) 3347, 2953, 2932, 2869, 1040 $cm^{-1}$; minor $R'_f$ 0.48 [$C_6H_6$-$Me_2CO$ (4:1)].

Compound **12** (5 mg) was dissolved in 10% ethanolic KOH (3 ml), and the mixture was stirred at 80° for 20 min. The mixture was cooled to 0° and the reaction quenched with 20% HCl. Most of the EtOH was removed; the residue was diluted with $H_2O$ and extracted with EtOAc. The organic extract was dried, concentrated, then purified by chromatography to give a single diol which was recrystallized from $CH_2Cl_2$/Skellysolve B: mp 138–140°; $R'_f$ 0.22 [$C_6H_6$-$Me_2CO$ (1:4)]; Ft-ir ($CHCl_3$ cast) 3345, 2952, 2933, 2869, 1045 $cm^{-1}$; cims ($NH_3$) $m/z$ $[M+NH_4]^+$ 238 (64); ms 220 (13), 205 (6), 202 (50), 179 (32), 166 (56), 148 (100), 120 (27), 111 (55), 109 (69), 108 (96), 95 (52), 55 (42).

ACETYLATION OF ARTHROSPOROL: 4-*O*-ACETYLARTHROSPOROL [**16**].—A solution of **3** (3.4 mg), $Ac_2O$ (5 drops), and $C_5H_5N$ (1.5 ml) was stirred at room temperature for 24 h. Workup in the usual way and purification by chromatography gave compound **16** which was recrystallized from Skellysolve B/$Et_2O$ (mp 150°).

4,8-*O,O*-DIACETYLARTHROSPOROL [**17**].—Arthrosporol [**3**] (4.7 mg), DMAP (catalytic amount), and $Ac_2O$ (5 drops) were mixed in $NEt_3$ and allowed to stand at room temperature for 3 days. The mixture was worked up in the usual way. Purification of the crude acetylate by chromatography gave compound **17**

as a waxy solid: $R'_f$ 0.86 [$C_6H_6$-$Me_2CO$ (2:7)]; Ft-ir ($CHCl_3$ cast) 3512, 2952, 1732, 1717, 1249, 1020 $cm^{-1}$; lreims 278 (3), 219 (50), 218 (100), 203 (13), 190 (17), 109 (30), 95 (17), 55 (14), 45 (58).

4,6,8-*O,O,O*-TRIACETYLARTHROSPOROL [**18**].—Arthrosporol [**3**] (2.4 mg) and *p*-toluenesulfonic acid were dissolved in $Ac_2O$ (1 ml), and the solution was stirred at room temperature for 3 h. The solution was diluted with $Et_2O$ (10 ml), washed (5% aqueous $NaHCO_3$, $H_2O$, brine), dried, and the solvent removed. The residue was chromatographed to give pure **18** as a viscous oil: $R'_f$ 1.43 [$C_6H_6$-$Me_2CO$ (1:8)]; Ft-ir ($CHCl_3$ cast) 1738, 1249, 1225, 1020 $cm^{-1}$; cims ($NH_3$) *m/z* $[M+NH_4]^+$ 398 (100); hreims 321 (1), 260 (11), 218 (8), 200 (100).

ACETYLDEHYDROARTHROSPORODIONE [**20**].—Compound **19** (4 mg), $C_5H_5N$ (1.5 ml), and $Ac_2O$ (4 drops) were stirred at room temperature for 12 h. The reaction mixture was worked up in the usual way. Purification by preparative tlc [$Me_2CO$-$C_6H_6$ (1:19)] gave pure **20**: $R'_f$ 1.07 [$C_6H_6$-$Me_2CO$ (1:8)]; $[\alpha]D$ −13.8° (*c* = 1.6, $CHCl_3$); Ft-ir ($CHCl_3$ cast) 1740, 1710, 1632, 1328, 1374, 1232 $cm^{-1}$; $^1H$ nmr ($CDCl_3$) δ 5.60 (1H, t, 2.5 Hz, H-9), 2.97 (1H, m, H-6), 2.87 (1H, ddd, 16, 9, 2.4 Hz, H-7α), 2.59 (1H, dd, 20, 9 Hz, H-9), 2.42 (1H, ddd, 19, 4, 1.5 Hz, H-5β), 2.40 (1H, qd, 7, 1.5 Hz, H-3), 2.25 (1H, ddd, 16, 7, 2.5 Hz, H-7β), 2.17 (3H, s, $CH_3CO$), 1.26 (3H, s, Me), 1.1 (3H, s, Me), 1.08 (3H, d, 7 Hz, H-15), 1.00 (3H, s, Me); $^{13}C$ nmr ($CDCl_3$) δ 217.5 (s, C-4), 204.9 (s, C-11), 181.4 (s, C-3), 170.3 (s, MeCO), 147.5 (s, C-1), 75.5 (d, C-9), 53.9 (s, C-10), 51.0 (d, C-3), 49.9 (d, C-6), 42.1 (s, C-2), 41.7 (t, C-5), 31.7 (t, C-7), 24.1 (q), 20.7 (q), 19.9 (q, *Me*CO), 18.6 (q), 9.4 (q); cims ($NH_3$) *m/z* $[2M+NH_4]^+$ 598 (0.3), $[M+NH_4]^+$ 308 (100); hreims (290.1512 calcd for $C_{17}H_{22}O_4$) found 290.1524 (7), 248 (89), 233 (100), 177 (25), 174 (119), 91 (19).

OXIDATION OF DEHYDROARTHROSPORODIONE.—Compound **19** (2.7 mg) was dissolved in $CH_2Cl_2$, and PCC (2.3 mg) was added. The yellow suspension was stirred at room temperature for 20 h. The suspension was filtered through florosil and the adsorbent washed with $CHCl_3$. The filtrate and washings were combined and concentrated. Purification of the residue by chromatography gave triketone **21** as a low melting solid: $R'_f$ 1.29 [$C_6H_6$-$Me_2CO$ (1:8)]; $[\alpha]D$ −153.9° (*c* = 0.7, $CHCl_3$); λ max (MeOH) 247 nm (ε = 4762); Ft-ir ($CHCl_3$ cast) 1736, 1696, 1616 $cm^{-1}$; $^1H$ nmr ($CDCl_3$) δ 3.10 (1H, dd, 17, 9 Hz, H-7α), 3.07 (1H, m, H-6), 2.74 (1H, dd, 18.5, 9 Hz, H-5α), 2.51 (1H, dd, 17, 6 Hz, H-7β), 2.39 (1H, m, H-3), 2.21 (1H, dd, 18.5, 5 Hz, H-5β), 1.23 (3H, s, Me), 1.19 (3H, s, Me), 1.16 (3H, s, Me), 1.15 (3H, d, 7 Hz, H-15); hreims (246.1251 calcd for $C_{15}H_{18}O_3$) found 246.1257 (100), 231 (7), 218 (3), 190 (29), 189 (53), 176 (50).

ACETYLATION OF COMPOUND **22**.—Compound **22** (1 mg), $C_5H_5N$ (1 ml), and $Ac_2O$ (2 drops) were stirred at room temperature for 24 h at which time the starting material was recovered unchanged. The solution was concentrated to dryness and the residue dissolved in $NEt_3$ (1 ml). $Ac_2O$ (2 drops) and DMAP (ca. 0.5 mg) were added and the mixture stirred overnight. Workup in the usual way gave a complex mixture (tlc). Purification by chromatography gave a small amount of a uv-active compound (compound **23**): Ft-ir ($CHCl_3$ cast) 1744, 1709, 1632, 1233 $cm^{-1}$; $^1H$ nmr ($CDCl_3$) δ 5.88 (1H, d, 2 Hz, H-5), 5.09 (1H, s, H-11), 3.97 (1H, dd, 7, 4 Hz, H-15α), 3.52 (1H, t, 8 Hz, H-8), 3.51 (1H, t, H-15β), 3.03 (1H, dd, 15, 9 Hz, H-7α), 2.84 (1H, ddd, 15, 8.5, 1.5 Hz, H-7β), 2.78 (1H, dd, 7, 4 Hz), 2.20 (3H, s, $CH_3CO$), 1.32 (3H, s, H-13), 1.27 (3H, s, H-12), 1.14 (3H, s, H-14).

## ACKNOWLEDGMENTS

We gratefully acknowledge the financial support provided by the Natural Sciences and Engineering Research Council of Canada. We thank Drs. A. Tsuneda and Y. Hiratsuka, Northern Forestry Centre, Edmonton, for bringing this problem to our attention and for providing culture of the fungus, Mrs. L. Sigler, University of Alberta Microfungus Collection, for identification of this fungus, Dr. J. Stalpers, Centraalbureau Voor Schimmelcultures, Baarn, Netherlands, for comments on the identity of the fungus, and Ms. A. Szenthe for help with the culture preparations.

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*Received 24 March 1989*