Tetrahedron Vol. 38, No. 10, pp. 1409 to 1412, 1982
Printed in Great Britain.

0040-4020/82/101409-04$03.00/0

METABOLITES OF BIRD'S NEST FUNGI. PART 18.† NEW OXYGENATED CADINANE DERIVATIVES FROM *CYATHUS STRIATUS*

WILLIAM A. AYER* and TORSTEN REFFSTRUP‡
Department of Chemistry, The University of Alberta, Edmonton, Alberta, Canada T6G 2G2

(*Received in U.S.A.* 22 *October* 1981)

Abstract—The bird's nest fungus *Cyathus striatus* Willd. ex Pers. when grown in aerated liquid culture produces three sesquiterpenes which are identified as schizandronol **1**, 7α - hydroxy - schizandronol **3** and schizandronol - 8,13β - oxide **5**. The identification is based on spectral investigation of the compounds and their derivatives. This is the first reported isolation of **1** from a fungal source. Compounds **3** and **5** have not been reported previously.

In our continuing investigation of the metabolites of bird's nest fungi (Nidulariaceae),[2] *Cyathus striatus* Willd. ex Pers. (strain CBS 169.37) has been investigated. When fermented in aerated culture for 15 days, *C. striatus* produces, among other compounds,[3] three sesquiterpenoids of the cadinane type which are the subject of this paper. The cadinane skeleton is of rather common occurrence in nature but the known cadinane derivatives are usually not as highly oxygenated as those reported herein.[1]

The sesquiterpenes were isolated from the culture broth by extraction with ethyl acetate and from the mycelium by extraction with acetone. The extract was subjected to gel filtration over Sephadex LH 20, then selected fractions were chromatographed over silica gel eluting with chloroform/ether or benzene/ether to give 7α-hydroxyschizandronol **3** and schizandronol-8,13β-oxide **5**. Schizandronol **1** was separated from selected fractions by column chromatography. Recrystallisation from ether/Skellysolve B gave **1** as white crystals of m.p. 105–106.5° and $[\alpha]_D^{24}$ −82°C (*c* 0.016, $CHCl_3$). Exact mass measurement established the molecular formula $C_{15}H_{22}O_2$. The identity of **1** with schizandronol was established by comparison of its physical properties (mp, IR, UV, ^{1}H NMR, ^{13}C NMR, MS) with those reported for **1** isolated from the tree Schizandra nigra Max.[4] The ORD spectrum of our material shows a negative Cotton effect, similar to that reported for schizandronol **1**, confirming the absolute stereochemistry. This constitutes the first report of schizandronol as a fungal metabolite.

The sesquiterpene **3** was isolated as white crystals, m.p. 151–154°. The composition of $C_{15}H_{22}O_3$ was established by HRMS. UV and IR data indicated that **3** is an α,β-unsaturated ketone (λ_{max}: 236 nm; ε 6,400; ν_{max}: 1667 cm^{-1}). The ^{1}H NMR spectrum of **3** shows resonances at δ 0.80 and 0.99 (two d's, each 3 H, J = 6.5 Hz) which were assigned to an isopropyl group in a chiral environment. Signals at δ4.94 and 4.61 indicate an exocyclic methylene group (IR ν_{max}: 900 cm^{-1}). A signal at δ4.22 (s, 2 H) was assigned to the methylene of a primary allylic alcohol and one at δ4.39 (dd, 1 H, J = 1 and 3 Hz) to an allylic secondary alcohol. In the diacetyl derivative **4** the corresponding resonances are observed at δ4.80 and 5.50 respectively.

Comparison of the above spectral data with those of **1** indicates that **3** is a hydroxy derivative of **1** and the only position possible for a secondary allylic hydroxyl is at C-7. The coupling constants observed for the C-7 proton indicate that the hydroxyl group is axial. This was confirmed by selective spin decoupling experiments on **3** which also established the *trans* ring fusion ($J_{4a,8a}$ = 12 Hz) and that the isopropyl group is equatorial ($J_{H5,H4a}$ = 11.5 Hz). The Cotton effect of **3** is negative and **3** therefore possesses the same absolute stereochemistry as **1**.

Compound **5**, isomeric with **3** but less polar, resisted crystallisation. The Cotton effect is negative. Again an α,β-unsaturated ketone is apparent (ν_{max}: 1673 cm^{-1}; λ_{max}: 235 nm, ε 6,200). The ^{1}H NMR is very similar to that of **1** except that the resonances attributable to the exocyclic methylene are missing. Instead signals at δ2.79 (dd) and 2.56 (d) are present. On acetylation, **5** formed a monoacetate **6** that has no free hydroxyl group (IR). Since **5** contains only one carbonyl group, the above data is consistent with the presence of an epoxide. Thus **5** appear to be the 8,13-oxide of schizandronol.

To confirm this structural assignment, and to determine the stereochemistry of the epoxide, we decided to attempt the preparation of both possible epoxides of O-acetylschandronol and examine their properties. Treatment of **2** with MCPBA (*m*-chloroperbenzoic acid) gave a mixture of isomers in the ratio of 4:1. The minor isomer was identical with **6**, confirming the constitution of the natural produc. Treatment of **2** with NBS (*N*-bromosuccinimide) in aq. acetone followed by treatment of the resulting bromohydrin with pyridine[5] provide a mixture of epoxides **6** and **7** in which **6** was now the major component (ratio 9:1). The main distinguishing feature in the ^{1}H NMR spectra of **6** and **7** is that **7** shows a simple AB quartet (J_{gem} = 4 Hz) centered at δ2.71 for the epoxide methylene protons, while in **6** one of the epoxide methylene protons (δ2.76) is further split (J's 4 (*gem*), 1 Hz). It was not possible, however, to locate the coupling partner. It is clear from the epoxidation reactions that there is a preferred direction of electrophilic attack on **2**. Examination of molecular models indicates that both α- and β- faces of **2** are relatively unhindered. In such cases, approach of bulky electrophilic reagents to an exocyclic double bond of a cyclohexane derivative usually occurs predominantly from the "equatorial" rather than an "axial" face.[6,7] The fact that the more sterically demanding hydrated "bromonium" ion gives

†For Part 17, see Ref. 1.

‡Present address: Department of Chemistry, Odense University, DK 5230, Odense M, Denmark.

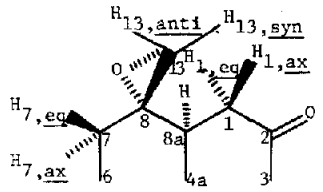


Figure 1

more stereoselectivity than MCPBA is in agreement with this view.[7] Thus the acetate of the natural epoxide **6** is assigned the β-oxide stereochemistry, and **7** is the α-oxide.

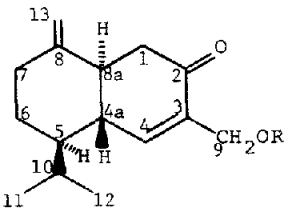
In the case of the acetate **4**, the α-substituent at C-7 might be expected to hinder attack from the α-face, and in the case of the bromohydrin sequence, favor formation of the α-epoxide **8**. Treatment of **4** with NBS in aq. acetone gave almost exclusively (>95%) epoxide **8**. A nuclear Overhauser experiment (NOE) showed a strong NOE effect between one of the epoxide protons (δ2.89, H_{13syn}, see Fig. 1) and the *axial* hydrogen at C-1 (10% enhancement), confirming the stereochemistry of the epoxide in **8**. Epoxidation of **4** with MCPBA provided, as the major product, the β-epoxide **9**. Interestingly, both **8** and **9** show simple AB quartets for the epoxide methylene protons, indicating that the long range coupling of one of the epoxide protons in **6** is to the *axial* proton at C-7.

EXPERIMENTAL

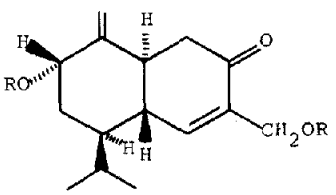
Melting points were measured on a Leitz microscope melting point apparatus and are uncorrected. Optical rotations were measured on a Perkin–Elmer 141 polarimeter. IR spectra were recorded as chloroform casts on a Nicolet 7199 FT spectrometer. Mass spectra were recorded on an A.E.I. MS-50 mass spectrometer coupled to a DS 50 computer and are given as *m/e* (rel. int.). NMR spectra were recorded on a Bruker WH 400 spectrometer, a Bruker WH 200 spectrometer, a Varian HA-100 spectrometer interfaced to a Digilab FT/NMR 3 data system, or a Bruker WP-60 spectrometer interfaced to a Nicolet 1080 computer.

Preparative tlc (ptlc) was performed on silica gel G 60 (E. Merck, Darmstadt) with a thickness of 0.5 mm, containg 1% electronic phorphor. The plates were visuallized by UV light or by spraying with 1% valillin in concentrated sulphuric acid. Skellysolve B refers to Skelly Oil Company light petroleum, b.p. 62–70°.

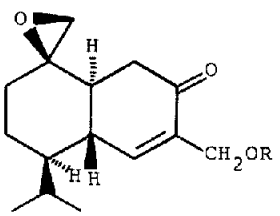
Mycelium from agar slants of *Cyathus striatus* Willd. ex Pers. (obtained from Centraalbureau voor Schimmelcultures, Baarn, the Netherlands, strain 169.37) was transferred to a 250 mL Erlenmeyer flask containing 150 mL of sterilised medium having the following composition per litre: maltose, 5.0 g; dextrose, 2.0 g; yeast extract (Difco), 2.0 g; KH_2PO_4, 0.5 g; $Ca(NO_3)_2$. 4 H_2O, 0.5 g; $MgSO_4$, 0.24 g; peptone, 0.2 g; asparagine, 0.2 g; $Fe_2(SO_4)_3$, trace: (Brodie's medium).[8] The flask was kept at 20–24° on a rotary shaker for 14 days. The contents of one such flask were used to inoculate 10 L of Brodie's medium which was then grown for 15 days at 22° on a New Brunswick Scientific microfermentation apparatus with stirring (200 rev./min.) and aeration (3 L/min.). To prevent foaming 1 mL of polypropylene glycol was added.



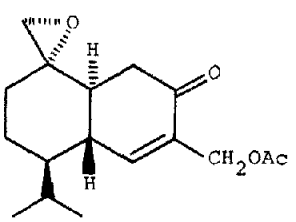
1 R = H
2 R = Ac



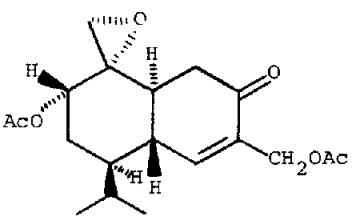
3 R = H
4 R = Ac



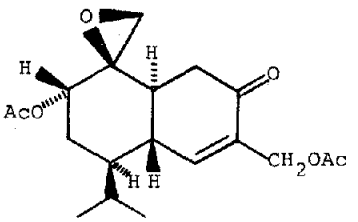
5 R = H
6 R = Ac



7



8



9

The mycelium was separated from the broth by filtration and was extracted with acetone in a Soxhlet extractor. The acetone extract was partitioned 3 times between a small volume of water and 10 volumes of ethyl acetate. The combined organic phases were evaporated to dryness giving 1.3 g/10 L fermentation. The extract from three 10 L fermentations were placed on a column of Sephadex LH 20 (200 g, length 70 cm) in methanol. Fractions of 10 mL were collected. Schizandronol **1** was eluted in fractions 18 and 19. Purification by column chromatography on SiO_2 with benzene-ether (100:0, 19:1, 9:1) gave pure schizandronol (in a yield of 8 mg/10 L fermentation).

The broth was extracted 5 times with 1/2 volume of ethyl acetate. The extracts were combined and evaporated to dryness. Yield, 1.8 g/10 L fermentation. The combined extracts from three 10 L fermentations were placed on a column of Sephadex LH 20 (200 g, length 70 cm.) in methanol and fractions of 10 ml were collected. Schizandronol **1**, 7α - hydroxyschizandronol **3** and schizandronol - 8,13β - oxide **5** were contained in fractions 17–19, and were separated by column chromatography on SiO_2 with $CHCl_3$–ether (100:0, 19:1, 9:1, 4:1). 10 mL fractions were collected. Schizandronol **1** eluted together with glochidonol[2] in fractions 11 and 12, schizandronol-8,13β-oxide **5** in fractions 16–21 and 7α-hydroxyschizandronol **3** in fractions 50–59.

Schizandronol **1**. Yield per 10 L fermentation: mycelium, 8 mg; broth, 4 mg. Recrystallization from ether/Skellysolve B gave white crystals, m.p. 105–106° (lit.[4] 110°, $[\alpha]_D^{24}$ −82° (*c* 0.016, $CHCl_3$) (lit.[4] $[\alpha]_D^{19}$ −105° (*c* 1.0, $CHCl_3$)). IR, UV, MS, ^{1}H NMR, ^{13}C NMR and ORD spectra were in perfect agreement with those reported.[4]

7α-Hydroxyschizandronol **3**. Yield per 10 L fermentation, 18 mg. Recrystallization from ethyl acetate gave brilliant white crystals of m.p. 151–154°. UV (MeOH) λ_{max}: 236 nm (ϵ 6,400); IR ($CHCl_3$): 3400, 2960, 1667, 1380, 1240, 900; $[\alpha]_D^{24}$ −26° (*c* 0.029, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$): δ7.05 (s, H-4), 4.94 (s, H-13), 4.61 (d, J = 1 Hz, H-13), 4.39 (dd, J = 3 and 1 Hz, H-7), 4.22 (s, 2H, H-9), 2.90 (distorted t, J ~ 13, 3 and 12 Hz, H-8a), 2.67 (dd, J = 3.5, 16 Hz, H-1*eq*), 2.43 (dd, J = 16, 13 Hz, H-1*ax*), 2.31 (m, H-10), 2.0 (complex, H-4a, H-5, H-6*eq*), 1.42 (ddd, J = 13.5, 12, 2.5 Hz, H-6*ax*), 0.99 and 0.80 (d, each 3 H, J = 6.5 Hz, H-11, H-12); MS *m/e* calc. for $C_{15}H_{22}O_3$: 250.1564; found: 250.1570; *m/e* 250 (78), 232 (27), 207 (35), 190 (34), 189 (81), 176 (10), 173 (50), 172 (17), 171 (32), 167 (57), 159 (31), 152 (24), 147 (58), 143 (51), 109 (38), 107 (48), 105 (70), 91 (100).

Schizandronol-8,13β-oxide **5**. Yield per 10 L fermentation, 6 mg. **5** was isolated as an oil. UV (MeOH) λ_{max}: 235 nm (ϵ 6,200); IR ($CHCl_3$): 3450, 2960, 2870, 1673, 1390, 1370; $[\alpha]_D^{24}$ −5°C (*c* 0.015, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$): δ7.09 (d, J = 0.8 Hz, H-4), 4.29 (d, 2 H, J = 0.8 Hz, H-9), 2.79 (dd, J = 4, 1.5 Hz, H-13), 2.56 (d, J = 4 Hz, H-13), 2.52 (dd, J = 15, 3 Hz, H-1*eq*), 2.3 (complex, 3 H), 1.9 (complex, 3 H), 1.4 (complex, 4 H), 1.02 and 0.85 (d, each 3 H, J = 7 Hz, H-11, H-12); MS *m/e* calcd. for $C_{15}H_{22}O_3$: 250.1564; found: 250.1571; *m/e* 250 (70), 232 (17), 220 (17), 219 (54), 207 (21), 189 (47), 147 (45), 105 (52), 93 (48), 91 (100).

Acetylschizandronol-8,13α-oxide **7**. *O*-Acetylschizandronol (**2**, 16 mg, 0.06 mmol) was treated with MCPBA (12 mg, 1.1 eq) in CH_2Cl_2 at 0° for 1 h, then warmed to room temperature and left 18 h. Conversion 50%. Ptlc with benzene–ether (3:1) gave at R_f = 0.4, 9 mg of a mixture containing the epoxides **6** and **7** in the ratio of 1:4, as determined by ^{1}H NMR. ^{1}H NMR (200 MHz, $CDCl_3$), α-isomer **7**: δ7.20 (s, 1 H), 4.75 (s, 2 H), 2.87 (d, 1 H, J = 4 Hz), 2.55 (d, 1 H, J = 4 Hz), 2.09 (s, 3 H), 1.03 (d, 3 H, J = 6.5 Hz), 0.89 (d, 3 H, J = 6.5 Hz).

Acetylschizandronol-8,13β-oxide **6**. Schizandronol-8,13β-oxide **5** was acetylated quantitatively with Ac_2O/pyridine. IR ($CHCl_3$): 2960, 2870, 1740 (s), 1680 (s), 1370, 1240, 1030. ^{1}H NMR (100 MHz, $CDCl_3$): 7.09 (s, 1 H), 4.74 (s, 2 H); 2.76 (dd, 1 H, J = 1, 4 Hz), 2.52 (d, 1 H, J = 4 Hz), 2.08 (s, 3 H), 1.01 (d, 3 H, J = 7 Hz), 0.85 (d, 3 H, J = 7 Hz).

O - *Acetylschizandronol - 8,13β - epoxide* **6** *from* **2**. *O*-Acetylschizandronol (**2**, 6.3 mg, 0.022 mmol) was dissolved in acetone (2 mL) and H_2O (0.3 mL) was added together with NBS (7.7 mg, 0.044 mmol). The mixture, when homogeneous, was kept dark at room temperature for 18 h. Tlc monitoring (benzene–ether (3:1)) showed complete conversion to the bromohydrin (MS, chemical ionization: *m/e* 352 for $C_{17}H_{25}O_4{}^{79}Br$). The reaction mixture was evaporated to dryness and then dissolved in CH_2Cl_2 (2 mL) and Al_2O_3 (neutral, 0.5 g) was added together with 3 drops of pyridine. This mixture was left for 72 h under occasional shaking and monitored by Tlc. The reaction mixture was filtered extracted with H_2O, dried and evaporated to dryness. Ptlc (benzene–ether (3:1)) gave the mixture of isomers **6** and **7** in the ratio of 9:1 as determined by NMR. Yield: 4.6 mg, 83%.

Diacetyl - 7α - hydroxyschizandronol **4**. 7α-Hydroxyschizandronol (10.2 mg, 0.04 mmol) was dissolved in Ac_2O and 5 drops of pyridine was added. After 4 hr the mixture was evaporated to dryness to give the title compound in quantitative yield. IR ($CHCl_3$): 2960, 1738 (s), 1687 (s), 1370, 1240 (s), 1025. ^{1}H NMR (400 MHz, $CDCl_3$): δ7.10 (s, 1 H), 5.50 (dd, 1 H, J = 3, 3.5 Hz), 5.15 (d, 1 H, J = 1 Hz), 4.82 (d, 1 H, J = 1 Hz), 4.79 (s, 2 H), 2.80 (distorted t, 1 H, J = 13.5, 12 and 3.5 Hz), 2.68 (dd, 1 H, J = 16, 3.5 Hz), 2.46 (dd, 1 H, J = 16 and 13.5 Hz), 2.28 (m, 1 H), 2.12 (s, 3 H), 2.08 (m, 3 H), 2.05 (s, 3 H), 1.82 (dddd, 1 H, J = 12, 12, 3.5 and 3 Hz), 1.48 (ddd, 1 H, 14, 13 and 3 Hz), 1.01 (d, 3 H, J = 7 Hz), 0.83 (d, 3 H, J = 7 Hz).

Diacetyl - 7α - hydroxyschizandronol-9,13α-oxide **8**. Diacetyl - 7α - hydroxyschizandronol (**4**, 13.6 mg, 0.04 mmol) in 4 mL of acetone and 0.4 mL of H_2O was treated with NBS (2 eq., 14.5 mg) for 18 h in the dark. The bromohydrin was quantitatively formed (tlc evidence). MS, chemical ionization: *m/e* 430 for $C_{19}H_{27}O_6{}^{79}Br$. The reaction mixture was evaporated to dryness and redissolved in CH_2Cl_2. Pyridine (5 drops) and Al_2O_3 (neutral 0.6 g) were added and the mixture was stirred at room temperature for 48 h. The reaction mixture was filtered, extracted with dil. HCl and H_2O, dried, and evaporated to dryness to give 10 mg, 71% of the title compound. IR ($CHCl_3$): 2960, 1740 (s), 1680 (s), 1380, 1240 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ7.11 (s, H-4), 4.78 (s, 2 H, H-9), 4.72 (dd, H-7, J = 2 and 4 Hz), 2.89 (d, H-13*syn*, J = 4 Hz), 2.81 (ddd, J = 14, 11 and 3.5 Hz, H-8a), 2.67 (d, H-13*anti*, J = 4 Hz), 2.50 (dd, H-1*eq*, J = 16 and 3 Hz), 2.30 (m, H-10 and H-4a), 2.15 (H-6*eq*, 2.07 (6 H, acetyl Me's), 1.94 (dd, H-1*ax*, J = 14 and 16 Hz), 1.72 (H-5*ax*), 1.52 (H-6*ax*), 1.0 (d, 3 H, J = 7 Hz), 0.86 (d, 3 H, J = 7 Hz).

Diacetyl - 7α - oxyschizandronol - 8,13β - oxide **9**. Diacetyl - 7α - hydroxyschizandronol (30.4 mg, 0.92 mmol) was treated with MCPBA (1.1 eq., 18 mg) in $CHCl_3$ overnight. Extraction with $NaHCO_3$ and H_2O followed by drying gave a mixture of isomers which was separated by ptlc (benzene-ether (3:1)). Isolation of the fraction showing R_f 0.5 after two elutions gave the title compound (9.5 mg, 30% yield). Diacetyl - 7α - oxyschizandronol - 8,13β - oxide **8** was isolated from the fraction having R_f 0.4 (4 mg, 14%). Compound **9** shows IR ($CHCl_3$): 2760, 1749, 1680, 1370, 1240, 1030 cm^{-1}. MS *m/e* calc. for $C_{19}H_{26}O_6$: 350.1729. ^{1}H NMR (200 MHz, $CDCl_3$): δ7.16 (s, H-4), 4.76 (s, H-9), 4.56 (dd, H-7*eq*, J = 1 and 2 Hz), 2.90 (d, H-13, J = 4 Hz), 2.81 (d, H-13, J = 4 Hz), 2.70 (m, 1 H), 2.45 (m, 2 H), 2.20 (d, 1 H, J = 3 Hz), 2.10 (6 H, acetyl Me's), 1.94 (dd, H-1*ax*, J = 10 and 13 Hz), 1.74 (m, 1 H), 1.07 (d, 3 H, J = 7 Hz), 0.88 (d, 3 H, J = 7 Hz).

Acknowledgements—We wish to express our appreciation of the interest shown by Dr. T. T. Nakashima and his associates at the NMR Laboratory of the University of Alberta. Drs. Peter P. Singer and R. H. McCaskill are thanked for stimulating discussions. The receipt of a travel grant from the Danish Natural Science Research Council (Grant No. 511-10009) and a grant from Provinsbankens Studielegat, Odense, is gratefully acknowledged, as is the financial support of the Natural Sciences and Engineering Research Council of Canada.

REFERENCES

[1]W. A. Ayer, R. H. McCaskill and T. Reffstrup, *Rev. Latinoamer. Quim.* in press.

[2]For a review of earlier work see W. A. Ayer, L. M. Browne, S. Fernández, D. E. Ward, and T. Yoshida, *Rev. Latinoamer. Quim.* **9**, 177 (1978).

[3]Several triterpenes have also been isolated from this strain of *C.*

striatus: W. A. Ayer, R. Flanagen, and T. Reffstrup, manuscript in preparation. Another strain of *C. striatus* yields a group of C_{25} compounds: H. J. Hecht, G. Höfle, W. Steglich, T. Anke, and F. Oberwinkler, *J. Chem. Soc. Chem. Commun.* 665 (1978).
[4]K. Takahashi and M. Takani, *Chem. Pharm. Bull.* **24**, 2000 (1976).
[5]H. Reich and T. Reichstein, *Helv. Chim. Acta.* **26**, 562, 747, 1799 (1943). These authors used *N*-bromoacetamide instead of *N*-bromosuccinimide.
[6]H. B. Henbest, *Proc. Chem. Soc.* 159 (1963).
[7]H. O. House, *Modern Synthetic Reactions*, 2nd Ed. W. A. Benjamin, Inc. Menlo Park, Calif. (1972), pp. 311–312.
[8]H. J. Brodie, *The Bird's Nest Fungi*, p. 36. University of Toronto Press, Toronto (1975).