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METABOLITES OF BIRD'S NEST FUNGI—VII†

BICYCLOFARNESANE SESQUITERPENES OF *MYCOCALIA RETICULATA* PETCH

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Abstract—The metabolites of the bird's nest fungus *Mycocalia reticulata* Petch have been examined and the bicyclofarnesane sesquiterpenes 7-ketodihydrodrimenin (**1**), 7β-hydroxydihydrodrimenin (**2a**), and 6α,7β-dihydroxydihydrodrimenin (**3a**) have been isolated. These compounds have not been obtained previously from natural sources, although **1** and **2a** are known transformation products of other natural products. Compound **3a** is new, and its structure was established by physical methods. The known triterpenoid glochidone (μ) and β-sitosterol were also isolated.

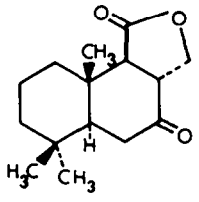
Liquid cultures of the small bird's nest fungi (Nidulariaceae) produce a variety of compounds. We have previously reported on the constituents of several species of this family (e.g. novel diterpenes of *Cyathus helenae* Brodie,[2] a degraded eudesmane-type sesquiterpene of *C. bulleri* Brodie,[3] and a new xanthone of *C. intermedius*[4]). In this paper, we describe the isolation and characterization of three related sesquiterpene lactones, **1**, **2a** and **3a** from cultures of the bird's nest fungus *Mycocalia reticulata* Petch.[5]

M. reticulata was grown in "still surface" culture on a liquid medium and the culture broth was extracted with ethyl acetate. The crude extract was separated into acidic and neutral fractions, and it is the latter fraction which is the subject of this report.

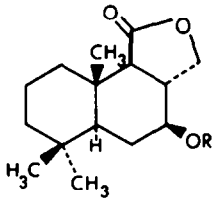
Preparative thin layer chromatography (ptlc) of the neutral fraction gave three major components. Crystallization of the least polar (highest R_f value) component gave needle-like crystals, m.p. 120–122°. The molecular formula, $C_{15}H_{22}O_3$‡ and the ^{1}HMR spectrum, which showed three methyl singlets, suggested a sesquiterpenoid. The IR spectrum shows carbonyl absorptions at 1770 and 1715 cm^{-1}, characteristic of a γ-lactone and a ketone respectively, thereby accounting for the three oxygen atoms present in the molecule. The mass spectrum shows a prominent peak at *m/e* 137 ($C_{10}H_{17}$). An *m/e* 137 fragment **6** is characteristic of higher terpenes containing the bicyclic system **4** which fragments as shown in Scheme 1.[6] This fragmentation, along with with the ^{1}HMR spectrum showing the three Me singlets, suggested that we were dealing with a bicyclofarnesane (drimane) type sesquiterpene (**7**).[7] Several of these are known to contain a γ-lactone moiety with the lactone CO located at either C-11 or C-12.[8] The ^{1}HMR spectrum shows a readily analyzable ABMX system for the pro-

†For Part VI. see Ref. 1.

‡The composition of all molecular and fragment ions reported in this paper were determined by high resolution mass spectrometry (hrms).



1



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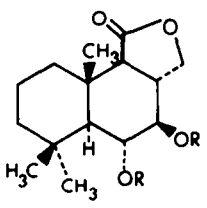
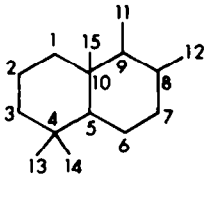
2b R=Ac



3a R=H
3b R=Ac



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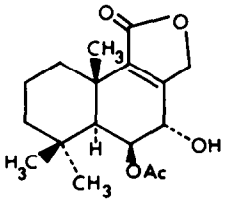

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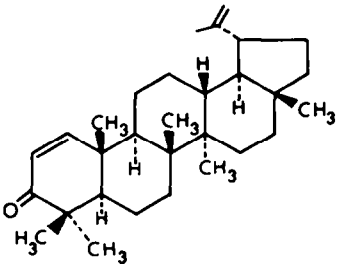

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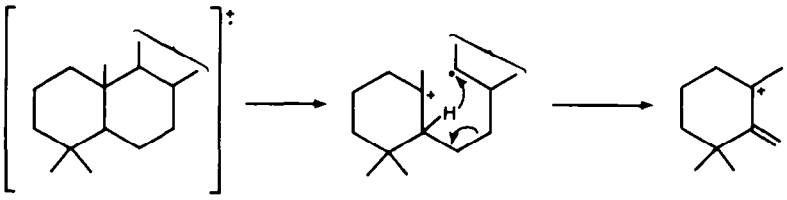

4 5 6

Scheme 1.

tons on C-12 (AB protons), C-8 (M proton), and C-9 (X proton) (details are given in the Experimental) which led to the tentative assignment of structure **1** (7-ketodihydrodrimenin)† to this metabolite. The ketolactone **1** had been obtained previously[8d] as a transformation product of ugandensolide (**8**). Comparison of the *M. reticulata* metabolite with an authentic sample of **1** revealed their identity.‡

The component of intermediate R_f value was obtained as fine needles, m.p. 157–158°, of molecular formula $C_{15}H_{24}O_3$ and proved to be the alcohol **2a**. Oxidation of the alcohol with Jones' reagent gave 7-ketodihydrodrimenin (**1**). Acetylation of **2a** afforded **2b**, the ^{1}HMR spectrum of which shows the signal for the C-7 hydrogen as a broad 8-line multiplet ($W_{1/2}$ = 26 Hz) indicative[10] of an axial hydrogen. 7β-Hydroxydihydrodrimenin (**2a**) has been synthesized from O-methylpodocarpane[9] and a comparison of the *M. reticulata* metabolite with an authentic sample of **2a** shows that they are identical.§ Neither **1** nor **2a** have been isolated previously from natural sources.

The component of lowest R_f value is a low melting (43–45°) solid of molecular formula $C_{15}H_{24}O_4$. Comparison of its IR, ^{1}HMR and mass spectra with those of **1** and **2a** suggested that this component is also a drimenin-type sesquiterpenoid. The IR spectrum shows γ-lactone absorption (1765 cm^{-1}) as well as OH absorption; the mass spectrum shows an ion $C_4H_5O_2$, also present in the mass spectrum of **1** and **2a** and presumably arising from the γ-lactone moiety; the ^{1}HMR shows the three Me singlets as well as multiplets at δ 4.32 (2 H), 3.15 (1 H) and 2.26 (1 H) characteristic of the 4 protons of the γ-lactone system. The mass spectrum does not however show a $C_{10}H_{17}$ peak (ion **6**, Scheme 1), but rather shows a $C_{10}H_{17}O$ peak, suggesting a OH substituted ion **6**. The metabolite was assigned structure **3a** (6α,7β-dihydroxydihydrodrimenin) after consideration of the properties of its 0,0-diacetyl derivative **3b**, prepared by treatment of **3a** with acetic anhydride-pyridine. The ^{1}HMR of **3b** revealed that the two protons (δ 3.88) *geminal* to OH groups in the diol were shifted downfield in the diacetyl derivative to δ5.57 and δ5.15. Each of these signals appears as a doublet of doublets, *mutually coupled by* 9 Hz. In addition the signal for the hydrogen at C-7 is coupled (9 Hz) to the hydrogen at C-8 (δ3.26), whereas the C-6 hydrogen is coupled (11.5 Hz) to a hydrogen resonating at about δ1.40, presumably the C-5 hydrogen.¶ The magnitude of the coupling constants observed (9–11.5 Hz) for the hydrogens at C-6 and C-7 indicates that all hydrogens involved (C-5, C-6, C-7, C-8) are axial hydrogens, in agreement with the stereochemistry shown in **3a** and **3b**. In addition, a doublet at δ2.33 in the spectrum of **3b** which is coupled (J = 9 Hz) to the C-8 signal, may be assigned to the hydrogen at C-9, which also must be axial (and thus α). Although sufficient material was not available to allow direct correlation of the previously undescribed **3a** with **1** or **2a**, it is reasonable to assume that the absolute configuration of **3a** is the same as **1** and **2a** and thus that **3a** represents the absolute stereochemistry of the diol.

Sesquiterpenes of the bicyclofarnesane skeleton **7** are found predominantly in trees and shrubs,[8,11] and have also been isolated from tobacco,[5b,12] liverwort,[13] and water pepper.[14] There are only two previous reports of their isolation from fungi, both of which are *Penicillium* species.[15]

In addition to the three sesquiterpenes described above, two minor components of the neutral metabolites of *M. reticulata*, isolated as described in the Experimental, have been identified as the triterpenoid glochidone (**9**)[16] and β-sitosterol. Glochidone has also been isolated from the mycelium extracts of the bird's nest fungi *Cyathus helenae* and *C. earlei*.‖

EXPERIMENTAL

Mass spectra were recorded on an A.E.I. MS-50 mass spectrometer coupled to a DS 50 computer, and are reported as *m/e* (relative intensity). Unless diagnostically significant only peaks at least 20% as intense as the base peak are reported. IR spectra were recorded on a Unicam SP1000 or Perkin–Elmer Model 421 dual grating spectrometer. NMR spectra were measured on a Varian HA-100 spectrometer interfaced to a Digilab FTS/NMR-3 data system or a Bruker WP-60 spectrometer interfaced to a Nicolet 1080 computer with TMS as internal standard. M.ps were recorded on a Thomas Model 40 micro hot stage or a Gallenkamp m.p. apparatus and are uncorrected.

Preparative tlc was carried out on 0.75 mm layers of silica gel G (W. Merck, Darmstadt) containing 1% electronic phosphor (General Electric, Cleveland). Unless otherwise specified the solvent system used to develop chromatograms was benzene-acetone-acetic acid (75:25:1). Skellysolve B refers to Skelly Oil Company light petroleum, b.p. 62–70°C.

Preliminary isolation of metabolites **1**, **1a** *and* **3a**. *Mycocalia reticulata* (strain 5465)†† was cultured as described for *Cyathus bulleri*[3] and after 30 days the culture broth was filtered and extracted with EtOAc. The EtOAc-extract was washed with water, dried ($MgSO_4$), and concentrated to provide the crude extract as a brown semi-solid (~0.2 g/l of culture broth). A methylene chloride solution of the crude extract (1.5 g) was

†The naming follows the system suggested by Wenkert and Strike.[9]

‡The authentic sample was kindly provided by Prof. C. J. W. Brooks.

†The authentic sample was kindly provided by Prof. E. Wenkert.

¶All ^{1}HMR assignments for **3b** are supported by the appropriate decoupling experiments.

‖Dr. L. M. Browne, private communication. We thank W. H. Hui for a sample of glochidone.[16b]

††Slant tube cultures were obtained from Dr. J. H. Ginns, Biosystematics Research Institute, Agriculture Canada, Ottawa, Ontario.

separated into acidic and neutral compounds by extraction with cold 5% NaOH aq. The neutral fraction (0.2 g) was subjected to ptlc to give nearly pure metabolites **1** (5 mg, R_f 0.78), **2a** (22 mg, R_f 0.55) and **3a** (44 mg, R_f 0.47).

7-Ketodihydrodrimenin, **1**. The least polar metabolite was recrystallized from Skellysolve B to give **1** as needles, m.p. 120–122°; $[\alpha]_D^{25}$ −118° (*c* 0.002, benzene). IR ($CHCl_3$): 1770, 1715, 1455, 1390, 1370, 1160, 1040 cm^{-1}; ^{1}HMR ($CDCl_3$): δ4.45 (m, 2, $W_{1/2}$ = 15 Hz, H-12), 3.28 (m, 1, $W_{1/2}$ = 30 Hz, H-8), 2.70 (d, 1, J = 12.5 Hz, H-9), 2.48 (m, 1, $W_{1/2}$ = 18 H, H-1β),† 2.45 (m, 2, $W_{1/2}$ = 16 Hz, H-6), 1.75 (m, 1, $W_{1/2}$ = 20 Hz, H-5), 0.91 (s, 6, CH_3), 0.85 (s, 3, CH_3); ms: *m/e* calcd. for $C_{15}H_{22}O_3$: 250.1569, found: 250.1569 (8), 235 (7), 194 (46), 166 (11), 137 (11), 124 (23), 123 (31), 122 (67), 109 (21), 85 (100), 69 (25). Metabolite **1** is identical (IR, ^{1}HMR, rotation, tlc, m.p.) with an authentic sample of 7-ketodihydrodrimenin.[8d].

7β-Hydroxydihydrodrimenin, **2a**. Compound **2a** was recrystallized from benzene–pentane to give fine needles, m.p. 157–158° (sealed tube), $[\alpha]_D^{25}$ −65° (*c* 0.0024, benzene). IR ($CHCl_3$): 3610, 3520, 1765, 1470, 1395, 1370, 1340, 1055, 1040, 1020 cm^{-1}; ^{1}HMR ($CDCl_3$): δ4.29 (m, 2, $W_{1/2}$ = 17 Hz, H-12), 4.11 (m, 1, $W_{1/2}$ = 26 Hz, H-7), 3.01 (m, 1, $W_{1/2}$ = 37 Hz, H-8), 2.23 (d, 1, J = 8.5 Hz, H-9), 2.17 (m, 1, H-1β), 1.82 (apparent ddd, 1, J = 13, 6, 2 Hz, H-5), 1.06 (s, 3, CH_3), 0.93 (s, 3, CH_3), 0.88 (s, 3, CH_3); ms: *m/e* calc. for $C_{15}H_{24}O_3$: 252.1726, found: 252.1708 (10), 237 (21), 234 (20), 219 (20), 196 (80), 178 (84), 167 (29), 137 (70), 124 (40), 123 (58), 119 (27), 109 (55), 107 (22), 105 (20), 95 (37), 93 (25), 91 (27), 85 (100), 81 (48), 79 (28), 77 (20), 69 (48), 67 (30), 55 (45). Metabolite **2a** is identical (tlc, IR, ^{1}HMR, rotation) with authentic 7β-hydroxydihydrodrimenin.[9]

6α,7β-Dihydroxydihydrodrimenin, **3a**. The most polar ptlc component was chromatographed again to give crystalline **3a**, m.p. 43–45°. IR ($CHCl_3$): 3580, 3430, 1765, 1465, 1390, 1370, 1335, 1070, 1020 cm^{-1}; ^{1}HMR ($CDCl_3$): δ4.32 (m, 2, $W_{1/2}$ = 22 Hz, H-12), 3.88 (m, 2, $W_{1/2}$ = 13 Hz, H-6, -7), 3.16 (m, 1, $W_{1/2}$ = 37 Hz, H-8), 2.26 (d, 1, J = 8.5 Hz, H-9), 2.15 (m, 1, H-1β), 1.19 (s, 3, CH_3), 1.10 (s, 6, CH_3); ms: *m/e* calc. for $C_{15}H_{24}O_4$: 268.1675, found: 268.1668 (6), 253 (7), 250 (13), 239 (20), 235 (15), 185 (47), 166 (70), 153 (100), 110 (63), 109 (40), 107 (31), 97 (53), 95 (26), 93 (37), 91 (29), 85 (52), 81 (40), 79 (31), 77 (24), 69 (54), 67 (32), 57 (20), 55 (53), 53 (22). Diol **3a** was further characterized as its diacetate derivative **3b**.

7β-Acetoxydihydrodrimenin, **2b**. A soln of alcohol **2a** (2.7 mg) in pyridine (0.1 ml) and Ac_2O (0.1 ml) was allowed to stand at room temp. for 1 day and then poured into water and extracted with ether (3×). The combined ethereal extracts were washed with water (2×), dried ($MgSO_4$), and concentrated under reduced pressure to give crude acetate (2.8 mg). Purification by ptlc yielded **2b** as an oil. IR ($CHCl_3$): 1770, 1740, 1470, 1385, 1370, 1335, 1260, 1135, 1025 cm^{-1}; ^{1}HMR ($CDCl_3$): δ5.12 (m, 1, $W_{1/2}$ = 26 Hz, H-7), 4.21 (m, 2, $W_{1/2}$ = 12 Hz, H-12), 3.10 (m, 1, $W_{1/2}$ = 37 Hz, H-8), 2.28 (d, 1, J = 8.5 Hz, H-9), 2.09 (m, 1, H-1β), 2.06 (s, 3, OAc), 1.90 (apparent ddd, 1, J = 13, 6, 2 Hz, H-5), 1.08 (s, 3, CH_3), 0.94 (s, 3, CH_3), 0.88 (s, 3, CH_3); ms: *m/e* calc. for $C_{17}H_{26}O_4$: 294.1831, found: 294.1835 (4), 279 (3), 234 (100), 219 (71), 178 (89), 137 (7), 123 (23), 119 (32), 109 (33), 107 (26), 105 (32), 95 (21), 93 (31), 91 (32), 85 (44), 81 (35), 79 (26), 69 (53), 67 (22), 55 (41).

6α,7β-Diacetoxydihydrodrimenin, **3b**. Acetylation of diol **3a** (30 mg) was performed as described for **2a**, followed by ptlc to give diacetate **3b** as an oil (15 mg) which partially solidified after several weeks. Recrystallization from cyclohexane-Skellysolve B yielded **3b** as needles, m.p. 122–123°; $[\alpha]_D^{25}$ −66° (*c* 0.016, benzene). IR ($CHCl_3$): 1770, 1745, 1470, 1370, 1345, 1270, 1140, 1040 cm^{-1}; ^{1}HMR ($CDCl_3$): δ5.51 (dd, 1, J = 11.5, 9 Hz, H-6), 5.15 (dd, 1, J = 9, 9 Hz, H-7), 4.24 (m, 2, $W_{1/2}$ = 14 Hz, H-12), 3.26 (m, 1, $W_{1/2}$ = 37 Hz, H-8), 2.33 (d, 1, J = 9 Hz, H-9), 2.15 (m, 1, H-1β), 2.04 (s, 3, OAc), 2.02 (s, 3, OAc), 1.41 (d, 1, J = 11.5 Hz, H-5), 1.20 (s, 3, CH_3), 1.05 (s, 3, CH_3), 0.94 (s, 3, CH_3); ms: *m/e* calc. for $C_{19}H_{28}O_6$: 352.1886. Found: 352.1882 (5), 292 (14), 250 (29), 235 (15), 232 (100), 217 (17), 195 (10), 153 (30), 149 (23), 85 (22), 82 (33), 69 (43), 55 (33). (Found: C, 64.33, H, 8.10%. Calc. for $C_{19}H_{28}O_6$: C, 64.75, H, 8.01%).

Jones' oxidation of 7β-hydroxydihydrodrimenin, **2a**. A soln of **2a** (2.0 mg) in acetone (0.5 ml) was stirred at room temp. and excess Jones' reagent (1 drop) was added. This was followed by addition of 2-propanol to destroy excess reagent. Solid $NaHCO_3$ was then added and the mixture stirred for a few min. Filtration through charcoal and removal of the solvents under reduced pressure gave a crystalline product (1.8 mg) which was shown to be identical with ketone **1** by comparison of tlc, IR, ^{1}HMR and ms data.

Isolation of glochidone (**9**) *and β-sitosterol*. The crude extract was separated into neutral and acidic fractions as described above. Column chromatography over silica gel (Woelm, <0.063 mm particle size) of the neutral fraction (0.4 g) employing gradient elution (chloroform to chloroform–methanol) gave in the early fractions components less polar than metabolites **1**, **2a** or **3a**. Further purification by ptlc (eluant chloroform–methanol, 50:1) of one component gave a very small amount (3 mg) of glochidone (**9**), R_f 0.55, which was identical (tlc, IR, ^{1}HMR and ms) with an authentic sample.

Another slightly more polar component was also subjected ptlc (eluant pentane–acetone, 4:1) to give a solid material (19 mg), R_f 0.7, which was recrystallized twice from Skelly solve B to give β-sitosterol, identified by comparison (tlc, IR, NMR and ms) with an authentic sample.

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REFERENCES

[1]W. A. Ayer, L. M. Browne and S. Fung, *Can. J. Chem.* **54**, 3276 (1976).
[2]W. A. Ayer and L. L. Carstens, *Ibid.* **51**, 3157 (1973); W. A. Ayer and H. Taube, *Ibid.* **51**, 3842 (1973).
[3]W. A. Ayer and M. G. Paice, *Ibid.* **54**, 910 (1976).
[4]W. A. Ayer and D. R. Taylor, *Ibid.* **54**, 1703 (1976).
[5]H. J. Brodie, *The Bird's Nest Fungi*, pp. 139–140. University of Toronto Press, Toronto (1975).
[6a]H. Budzikiewicz, C. Djerassi and D. H. Williams, *Structural Elucidation of Natural Products by Mass Spectrometry*, Vol. 2, pp. 155–164. Holden-Day, San Francisco (1964); [b]J. R. Hlubucek, A. J. Aasen, S.-O. Almquist and C. R. Enzell, *Acta. Chem. Scand.* **B28**, 289 (1974).
[7]T. K. Devon and A. I. Scott, *Handbook of Naturally Occurring Compounds*, Vol. 2, pp. 58–72. Academic Press, New York (1972).
[8a]C. Djerassi and S. Burnstein, *Tetrahedron* **7**, 37 (1959); [b]L. Canonica, A. Corbella, P. Gariboldi, G. Jommi, J. Křepinský, G. Ferrari and C. Casagrande, *Ibid.* **25**, 3895, 3903 (1969); [c]H. H. Appel, J. D. Connolly, K. H. Overton and R. P. M. Bond, *J. Chem. Soc.* 4685 (1960); [d]C. J. W. Brooks and G. H. Draffan, *Tetrahedron* **25**, 2887 (1969).
[9]E. Wenkert and D. P. Strike, *J. Am. Chem. Soc.* **86**, 2044 (1964).
[10]A. Hassner and C. Heathcock, *J. Org. Chem.* **29**, 1350 (1964).
[11]C. Djerassi, P. Sengupta, J. Herran and F. Walls, *J. Am. Chem. Soc.* **76**, 2966 (1954); L. Caglioti, H. Noef, D. Arigoni and O. Jeger, *Helv. Chim. Acta* **41**, 2278 (1958); H. H. Appel, C. J. W. Brooks and K. H. Overton, *J. Chem. Soc.* 3322 (1959); *J. W. Loder, Aust. J. Chem* **15**, 389 (1962); H. H. Appel, R. P. M. Bond and K. H. Overton, *Tetrahedron* **19**, 635 (1963); I. Kubo, Y.-W. Lee, M. Pettei, F. Pilkiewicz and K. Nakanishi, *J. Chem. Soc.* Chem. Commun. 1013 (1976).
[12]A. J. Aasen, C. H. G. Vogt and C. R. Enzell, *Acta Chem. Scand.* **B29**, 51 (1975).

†This signal is partially hidden by the 4-line multiplet centered at δ2.45 (H-6). However, in the spectrum in C_6D_6 the H-6 signal is shifted upfield by 0.3 ppm, exposing the H-1β signal which appears as a doublet of double doublets (J = 11, 4, 2 Hz). The low field position of the H-1β signal is presumably caused by the deshielding effect of the C-11 carbonyl and is well documented in the steroid field.[17]

[13]S. Huneck, *Z. Naturforschg.* **22b**, 462 (1967).
[14]C. S. Barnes and J. W. Loder, *Aust. J. Chem.* **15**, 322 (1962).
[15]T. J. King, J. C. Roberts and D. J. Thompson, *J. Chem. Soc.* Perkin Trans. I, 78 (1973); and ref. 9 cited; C. H. Calzadilla, G. Ferguson, S. A. Hutchinson and N. J. McCorkindale, *5th Int. IUPAC Symp. Chem. Natural Products.* London, Abstracts, 287 (1968).
[16a]A. K. Ganguly, T. R. Govindochari, P. A. Mohamed, A. D. Rahimtulla and N. Viswanathan, *Tetrahedron* **22**, 1513 (1966); [b]W. H. Hui and M. L. Fung, *J. Chem. Soc.* (C), 1710 (1969).
[17]N. S. Bhacca and D. H. Williams, *Applications of NMR Spectroscopy in Organic Chemistry*, pp. 66–69. Holden–Day, San Francisco (1964).