Pergamon

Tetrahedron Letters 39 (1998) 6073–6076

TETRAHEDRON LETTERS

New Nardosinane and Aristolane Sesquiterpenes from the Fruiting Bodies of *Russula lepida* [1]

Giovanni Vidari,* Zongling Che and Luigi Garlaschelli

Dipartimento di Chimica Organica, Universita' di Pavia, Via Taramelli 10, 27100 Pavia, Italy

Received 19 May 1998; accepted 15 June 1998

Abstract

Three new naturally occurring sesquiterpenes, rulepidanol and rulepidadienes A and B were isolated from the fruiting bodies of *Russula lepida*. Their structures were established by spectroscopic methods. These compounds together with aristolone are the first nardosinane and aristolane sesquiterpenes isolated from Basidiomycetes.

Keywords: Terpenes and terpenoids; Fungi; Natural products; NMR

The Russulaceae family is one of the largest in the subdivision Basidiomycotina in Whitthaker's Kingdom of Fungi [1] and comprises hundreds of species, worldwide distributed, belonging to the genera *Lactarius* and *Russula*. While secondary metabolites occurring in the fruiting bodies of European *Lactarius* species have thoroughly been investigated [2], the *Russula* mushrooms have received less attention, notwithstanding the larger number of existing species. So far, lactarane and secolactarane sesquiterpenes have been isolated from *R. sardonia* [3] and *R. queletii* [4] , lactaranes have been reported from *R. emetica* [5] and from *R. brevipes* [6], protoilludane sesquiterpenes have been found in *R. delica* [7, 8], while velutinal esters and related marasmane derivatives have been isolated from *R. queletii* [4], *R. cuprea* [9], *R. atropurpurea* [10], *R. mairei* [10], and *R. foetens* [10]. These partial data seem to indicate that most acrid and hot pungent tasting Russulaceae species are very similar to each other not only for many morphological features, but also for the chemical contents of sesquiterpenoids. Furthermore, all sesquiterpenoids isolated so far from *Russula* species are believed to be biosynthesized from protoilludane precursors, which through skeleton rearrangements give rise to the basic structures of the other classes displayed in Fig 1. Therefore, it is interesting to investigate those *Russula* species which may contain sesquiterpenes arising from a different biosynthetic pathway. In this context we examined *Russula lepida* Fr. (syn. *R. rosacea* (Pers. ex) Gray), a species which does not respond to the so-called "sulphovanillic mixture" [11]. This simple test, used in Mycology for mushroom identification, indicates the occurrence of velutinal esters and the derived marasmane- lactarane group of sesquiterpenes when a black-blue colour develops in the cold on mushrooms cystidia exposed to a solution of vanillin in H_2SO_4 [12]. Moreover, different authors describe the taste of *R. lepida* as slightly bitter or minty-resinous, but never acrid or pungent.

---

[1] Part 43 of the series "Fungal Metabolites"

* Corresponding Author. E-mail: vidari@chifis.unipv.it

0040-4039/98/$ - see front matter
PII: S0040-4039(98)01251-9

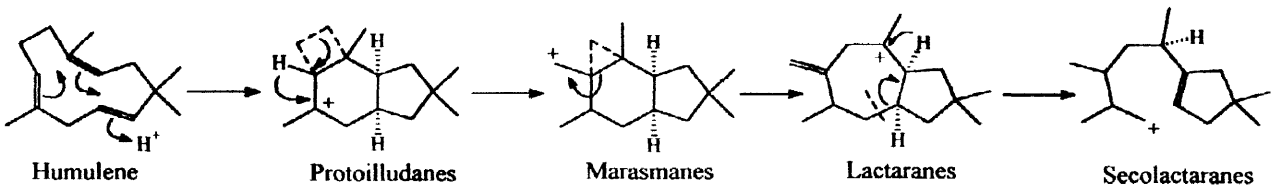
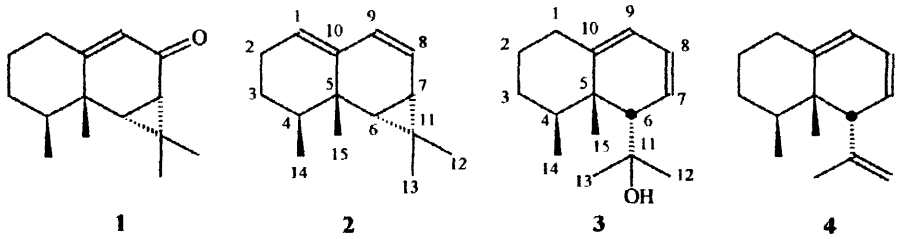


Fig 1

1 Kg of apparently undamaged fruiting bodies of *R. lepida* were frozen at - 20 °C and extracted with CH_2Cl_2 in the cold. The residue (4 g) was eluted with EtOAc on an activity III Al_2O_3 column to remove free carboxylic group compounds, mainly fatty acids. The neutral fraction was further separated on several RP18 columns (MeOH: EtOAc, 9:1) and on Si gel centrifugal circular chromatographic plates (Chromatotron) to yield eventually **1** (20 mg), **2** (3 mg), **3** (8 mg), **4** (2 mg). Compound **1** was found to be identical to (+)-aristolone (α-ferulone) by comparing mp, α_D, UV, ^1H and ^{13}C NMR spectra with literature values [13, 14].



Compound **2**, [2] $C_{15}H_{22}$ had five double bonds equivalents. A UV absorption band at 248 nm, along with four sp^2 carbons in the ^{13}C NMR, and three olefinic methine protons in the ^1H NMR spectrum, indicated the presence of a conjugated diene. The methine at one extreme of this system was further coupled with a methylene group, which was also coupled with a $CH_2CH(Me)$-C_{quat} spin system; the methine at the opposite end was further coupled to a CH (C_{quat})CH(C_{quat}) group. These data, together with the presence of three additional methyl groups attached to two quaternary sp^3 carbons were best accomodated by an aristoladiene structure in which the conjugated double bonds were connecting C-1 with C-8, as in formula **2**.

Rulepidanol **3** [3] showed in its ^{13}C NMR spectrum three sp^2 methine and one sp^2 quaternary carbons which were assigned to a homoannular diene system (λ_{max} 268 nm). Therefore, considering that the molecular formula was established as $C_{15}H_{24}O$ [CIMS ($[M+H]^+$ 221) along with ^{13}C NMR DEPT data (Table 1)], compound **3** was bicyclic. ^1H -^1H COSY and ^1H-^{13}C COSY spectra permitted establishment of two proton systems, one at C-1 through C-14, and another at C-9 through C-6. To complete the structure of **3**, three isolated spin systems, i. e. one quaternary methyl [δ 0.96 (s)] and two methyl groups geminal to a tertiary hydroxyl group [δ_C 77.1 (0)] , had to be taken into account. The connectivity of these units was assigned on the basis of COLOC correlations (see fig. 2A). Two or three-bond couplings from H-1 to C-10, from H-9 to C-10, from H-4 to C-5 and C-15, and from H-6 to C-5 allowed to establish the

---

[2] Colorless oil; ^1H and ^{13}C NMR spectra, Table 1; UV ($CHCl_3$) λ_{max} nm (log ε) 248 (3.82); IR ν^{liquid}_{max} 3030, 2960, 2930, 1640, 1610, 1460, 1378, 1365, 975, 850, 830 cm^{-1} ; GCMS *m/z* (rel. int.) 202 (M^+, 78), 187 ($[M-Me]^+$, 63), 173 (7), 159 ($[M - C_3H_7]^+$, 100), 145 (92), 131 (67), 117 (64), 105 (45), 91 (57), 77 (27), 65 (12), 55 (15), 41 (31)

[3] Colorless oil; $[\alpha]^{20}_D$ = + 84.1 (c = 0.6, $CHCl_3$); $CD_{252\,nm}$ ($CHCl_3$) Δε + 1.2; ^1H and ^{13}C NMR spectra, Table 1; UV (EtOH) λ_{max} nm (log ε) 268 (3.59); IR ν^{liquid}_{max} 3400 (sharp), 3040, 2970, 2925, 1455, 1425, 1380, 1268, 1140, 960, 870, 784 cm^{-1} ; CIMS (CH_4) *m/z* 221 $[M + H]^+$.

molecular structure of rulepidanol as **3**. The relative configurations of C-4, C-5, and C-6 stereocenters were established by NOESY spectroscopy (see Fig. 2B): the important nOe correlations were between H-6 and H_3-14 and H_3-15, between H-4 and H_3-13, and between αH-1 and H_3-13, indicating the stereochemistry shown in formula **3**.

In the CD spectrum of **3** a positive CE for the $\pi \rightarrow \pi^*$ transition of the skewed conjugated diene was observed, indicating P helicity [15] and thus that the absolute configuration of rulepidanol could be assigned as 4*S*, 5*S*, 6*S*.

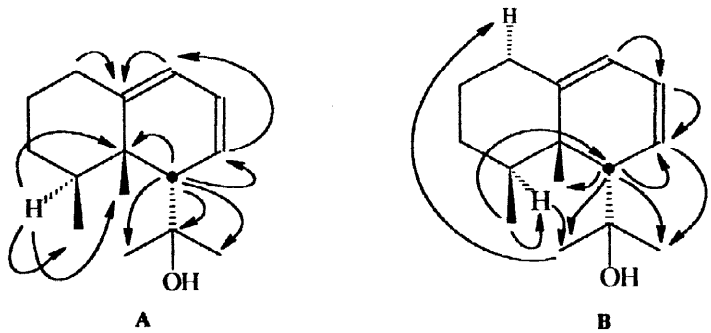


Fig 2. Pertinent COLOC (A) and NOESY (B) correlations observed for rulepidanol **3**

Table 1
^1H [a] and ^{13}C [b] NMR Data for compounds **2**, **3**, and **4** in $CDCl_3$

	2		**3**		**4**	
	^{13}C	^1H	^{13}C	^1H	^{13}C	^1H
1	121.9 (1)	5.26 brt 4.0 0.8	32.4 (2)	2.29 m (βH) 2.32 m (αH)	32.1 [g] (2)	2.20 - 2.28 m
2	25.1 (2)	2.11-2.20 m	24.7 (2)	1.40 m 1.68 m	25.6 (2)	1.25 -1.72 m
3	27.6 (2)	1.47-1.70 m	32.0 (2)	1.31 m (βH) 1.60 m (αH)	31.6 [g] (2)	
4	35.7 (1)	1.65 m	34.6 (1)	2.54 ddq 12.0, 4.0, 6.5	35.2 (1)	
5	34.5 (0)	-	41.7 (0)	-	39.5 (0)	-
6	34.4 (1)	0.92 d 8.5	51.4 (1)	2.18 d 6.5	52.8 (1)	2.83 brd 6.0
7	24.3 (1)	1.15 dd 8.5 5.3	125.9 (1)	5.59 ddt 9.5, 6.5 ,1.2	124.9 (1)	5.43 ddq 9.5 6.0 1.0
8	124.2 (1)	5.72 ddq 10.0 5.3 0.8	123.4 (1)	5.82 ddt 9.5, 5.2, 0.8	123.2 (1)	5.83 brdd 9.5 5.3 0.7
9	126.6 (1)	5.83 dq 10.0 0.8	117.6 (1)	5.47 ddt 5.2, 2.5, 1.2	117.6 (1)	5.47 m
10	141.6 (0)	-	147.3 (0)	-	146.4 [h] (0)	-
11	26.2 (0)	-	77.1 (0)	-	145.6 [h] (0)	-
12	14.6 [c] (3)	0.90 [d] s	25.9 [e] (3)	1.24 s	113.7 (2)	4.62 m 4.78 brd 2.5
13	22.9 [c] (3)	0.95 [d] s	31.5 [e] (3)	1.26 s	18.5 (3)	1.71 dd 1.5 0.8
14	15.8 (3)	0.98 d 6.5	18.0 [f] (3)	0.94 d 6.5	17.0 (3)	0.84 d 6.5
15	29.2 [c] (3)	1.11 [d] s	18.1 [f] (3)	0.96 s	17.4 (3)	0.95 s
OH	-	-	-	1.60 s	-	-

[a] 300 MHz; δ_H values in ppm from TMS, *J* in Hz.
[b] 75.5 MHz; δ_c values in ppm relative to $CDCl_3$ at 77.0. The numbers in parentheses indicate the number of hydrogens attributed to the corresponding carbon and were determined from DEPT experiments.
[c-h] Attributions can be interchanged.

The structure of rulepidadiene B 4[4] was easily assigned by comparison of its NMR data with those of compound **3**, which showed the signals of a 2-propenyl group (δ 1.71 ppm, olefinic Me; δ 4.62 and 4.78 ppm, >C=CH_2) instead of a 2-hydroxypropyl group.

Nardosinane sesquiterpenes are believed to derive in Nature from an aristolane precursor [16]. The co-occurrence of compounds **3** and **4** with **1** and **2** in the same mushroom reinforces this hypothesis and suggests that the absolute configurations of the two olefins **2** and **4** correspond to those of **1** and **3**, respectively.

Aristolane and nardosinane sesquiterpenes are of a type rather rare in Nature; they have been isolated both from terrestrial plants and marine organisms [17]. This is the first finding of members of these classes in a fungal species. It is of evolutionistic significance the elaboration by *Russula lepida* of nardosinane and aristolane sesquiterpenes antipodal to those usually found in higher plants and belonging, instead, to the enantiomeric series typical of several liverworts and Octocorallia.

Acknowledgements

We thank Alfredo Gatti of the Mycological group in Voghera (Italy) for collecting and identifying the mushrooms, the Italian CNR and MURST (ex 40%), and the EU Commission (Contract No ERBFAIRCT961781) for financial support. Z. Che was on leave of absence from the Fujian Institute of Testing Technology (Fuzhou, China).

References

[1] Witthaker R H. Science 1969;163:150-160.

[2] Vidari G, Vita-Finzi P. Sesquiterpenes and Other Secondary Metabolites of Genus *Lactarius* (Basidiomycetes): Chemistry and Biological Activity. In: Atta-ur-Rahman, editor. Studies in Natural Product Chemistry. Vol 17. Amsterdam: Elsevier, 1995:153-206.

[3] Andena A, De Bernardi M, Del Vecchio A, Fronza G, Mellerio G, Vidari G, Vita-Finzi P. Phytochemistry. 1980;19: 93-97

[4] Sterner O, Bergman R, Franzén C, Wickberg B. Tetrahedron Lett. 1985;26:3163-3166.

[5] Kobata K, Kano S, Shibata H. Biosci. Biotechnol. Biochem. 1995;59:316-318.

[6] Suri OP, Shah R, Satti NK, Suri KA. Phytochemistry 1997;45:1453-1455.

[7] Clericuzio M, Fu J, Pan F, Pang Z, Sterner O. Tetrahedron 1997;53:9735-9740.

[8] Clericuzio M, Pan F, Han F, Pang Z, Sterner O. Tetrahedron Lett. 1997;38:8237-8240.

[9] Clericuzio M, Sterner O. Phytochemistry 1997;45:1569-1572.

[10] Vidari G. unpublished results.

[11] Marchand A. Champignons du Nord et du Midi - Tome 5, Les Russules. Hachette, 1977:72-73.

[12] Gluchoff-Fiasson K, Kühner R. C. R. Acad. Sci. Ser III. 1982;294:1067-1071.

[13] Piers E, Britton RW, de Waal W. Can. J. Chem. 1969;47:831-840.

[14] Harrigan GG, Ahmad A, Baj N, Glass TE, Gunatilaka AAL, Kingston DGI. J. Nat. Prod. 1993;56:921-925.

[15] Eliel E, Wilen SH. Stereochemistry of Organic Compounds. New York : John Wiley, 1994:1038-1043.

[16] Rucker G, Frey K. Liebigs Ann. Chem. 1997:389-392 and references cited therein.

[17] Connolly JD, Hill RA. Dictionary of Terpenoids Vol. 1. London: Chapman and Hall, 1991: 420-423.

---

[4] Colourless oil; ^1H and ^{13}C NMR spectra, Table 1; UV (EtOH) λ_{max} nm (log ε) 259 (3.62); IR ν^{liquid}_{max} 3070, 3030, 2960, 2930, 1610, 1450, 1375, 1260, 1210, 1150, 890, 760, 730 cm^{-1} ; GCMS *m/z* (rel. int.) 202 (M^+, 100), 187 (61), 173 (11), 159 (34), 145 (60), 131 (71), 119 (30), 117 (32), 105 (68), 95 (25), 93 (42), 91 (65), 81 (31), 77 (32), 41 (42).