Constituents of Higher Fungi. Part VIII (1). Isolactarorufin, a Novel Tetracyclic Sesquiterpene Lactone from Lactarius Rufus

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Lactarius rufus extracts yielded along with tricyclic lactarorufins a novel tetracyclic sesquiterpene lactone with cyclopropane ring condensed with lactone ring - isolactarorufin (1). Its structure was established on the basis of chemical transformations and full spectral analysis.

Isolactarorufin $C_{15}H_{22}O_4$ (1), mp. 181°, $[\alpha]_D^{20}$ = +8.4° (in EtOH, c ~1) was isolated from ethanolic extracts of Lactarius rufus by standard procedure along with other lactarane derivatives (2,3) in very low yield.

It is isomeric with lactarorufin A (8); however, it differs from the latter by the lack of significant UV absorption above 200 nm and by higher value of IR lactonic carbonyl frequency (1768 cm^{-1}), thus showing to be a saturated γ-lactone. The PMR, ^{13}C-NMR as well as IR spectra of isolactarorufin point to its full saturation, hence it must have a tetracyclic structure. In the IR spectrum a strong band at 3600 cm^{-1} (hydroxyl groups) is visible, and the presence of two alcoholic groups was also confirmed by the PMR spectrum of 1 measured in pyridine-D_5, exhibiting a diprotonic hydroxyl-proton signal at 6.34 ppm. Isolactarorufin could be easily acetylated at room temperature to

the monoacetate $C_{17}H_{24}O_5$ (2), whose IR spectrum still contained a hydroxyl band at 3500 cm^{-1}.

These facts prove that isolactararufin (1) is like lactararufin A (8) a dihydroxy-γ-lactone.

The PMR spectrum of isolactararufin reveals further similarity with lactararufin A. It exhibits three methyl group signals at 0.98, 1.12 and 1.90 ppm (in pyridine-D_5). The first two signals were ascribed to two geminal methyl groups, the third one to the methyl group on carbon atom bearing the tertiary hydroxyl group, since this signal was shifted downfield in the dehydrated products 5 and 6. The broad singlet at 4.52 ppm was assigned to the methylene protons in γ-lactone ring; as it can be expected this signal becomes a very well resolved quartet in the PMR spectrum of the monoacetate 2. Its large coupling constant J_{AB} = 10 Hz is very characteristic for saturated γ--lactones of this type (4). The doublet at 4.78 ppm was ascribed to H-8, which was further substantiated by its downfield shift in the PMR spectrum of the acetate 2. The relatively large coupling constant J = 9 Hz demonstrates the trans relationship between H-8 and the bridge proton H-9. In the PMR spectrum of the synthetic isomer epi-isolactararufin (1a) the signal of H-8 is a broad singlet, proving the cis relationship between H-8 and H-9. The remaining PMR signals were overlapping themselves and even the PMR spectrum of isolactararufin acetate measured with the addition of Eu(dpm)$_3$ did not permit further structural assignments.

The most important feature of the ^{13}C NMR spectrum of 1 were the signals of three carbon bonded quaternary carbon atoms at 38.7, 33.9, 38.2 ppm. The first one was assigned to C-11 with two geminal methyl groups, the next one to C-7, as the

lactonic methylene protons in the PMR spectrum were split only by the geminal coupling and the H-8 signal was a doublet (coupled with H-9). The last signal was assigned to C-6 since the PMR did not show the presence of any proton in position α to the carbonyl at C-4. The ^{13}C NMR spectrum of 1 revealed further the presence of four methylene groups; consequently one of them i.e. that at the highest field (18.1 ppm) had to be assigned to cyclopropane methylene carbon atom C-5. The geminal coupling constant of H-5 protons was found to be 5.5 Hz in the well resolved spectrum of the dehydrated compound 5 measured in the presence of Eu(fod)$_3$, which is in excellent agreement with the known (5) literature data (Fig. 1). Thus the structure of the lactonic part and its environment of isolactarorufin was established - we assumed that the hydrocarbon part of isolactarorufin was the same as that of lactarorufin A (8) as both must have the same biogenetic origin, and also because the ^{13}C NMR signals of this part in the relevant lactaranes (6) are practically identical as illustrated in the Table I.

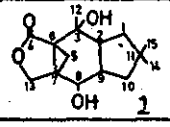
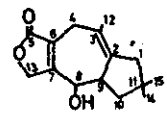
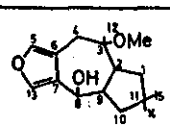
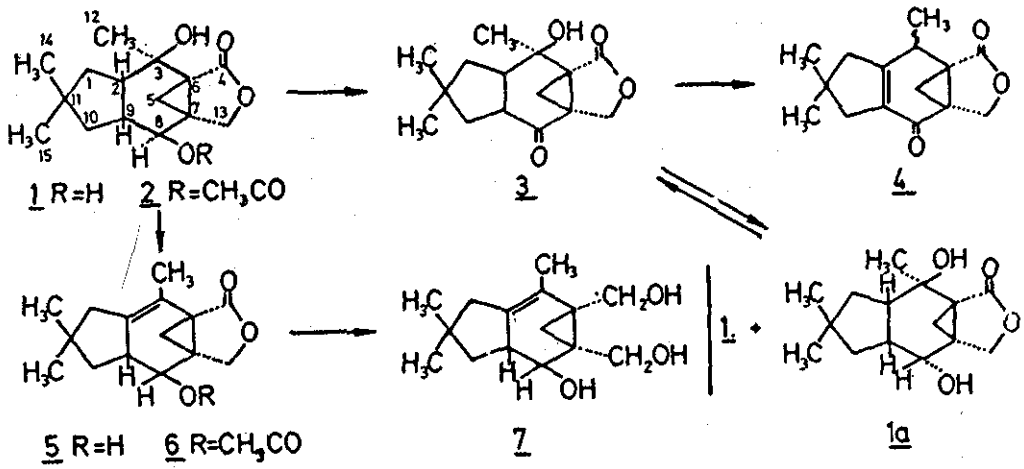
Nº C	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	40.7 t	47.0 d	70.8 s	175.3 s	18.1 t	33.9 s	38.2 s	74.8 d	43.6 d	48.1 t	38.7 s	23.9 q	72.7 t	28.8 q	26.7 q
	46.8 t	124.0 s	136.3 s	30.2 t	161.5 s	126.9 s	112.8 s	71.1 d	48.0 d	45.5 t	37.0 s	22.5 q	70.7 t	27.1 q	28.6 q
	45.3 t	46.8 d	80.2 s	28.0 t	139.7 d	127.3 s	127.3 s	66.7 d	46.8 d	45.3 t	36.6 s	24.4 q	141.8 d	29.9 q	27.7 q

Table 1

The chemical reactions confirmed this structural working

hypothesis. The action of thionyl chloride in pyridine converted isolactarorufin acetate (2) into the olefinic compound 6 with tetrasubstituted double bond. The hydrolysis of the acetoxy group yielded the free alcohol 5, whose Eu(fod)$_3$ shifted PMR spectrum gave the final evidence of the existence of cyclopropane ring as mentioned above. Jones oxidation of 1 produced the keto-derivative 3 and its dehydration with thionyl chloride in pyridine gave the α,β-unsaturated ketone 4 with a UV absorption at 237 and 255 nm.



Scheme 1

LAH reduction of the new acetate 6 gave the triol 7, whose PMR spectrum revealed very clearly the signal of H_b-5 proton (cyclopropyl methylene) at 0.75 ppm (geminal coupling constant with H_a-5, J=5 Hz); H_a-5 proton signal was found at 1.34 ppm overlapping with other signals (Scheme 1).

Fig. I presents the best resolved, Eu(fod)$_3$ shifted PMR spectrum of dehydrated isolactarorufin (5), where signals of all protons are clearly visible. The structural assignments

shown on the figure were confirmed by all possible decoupling experiments.

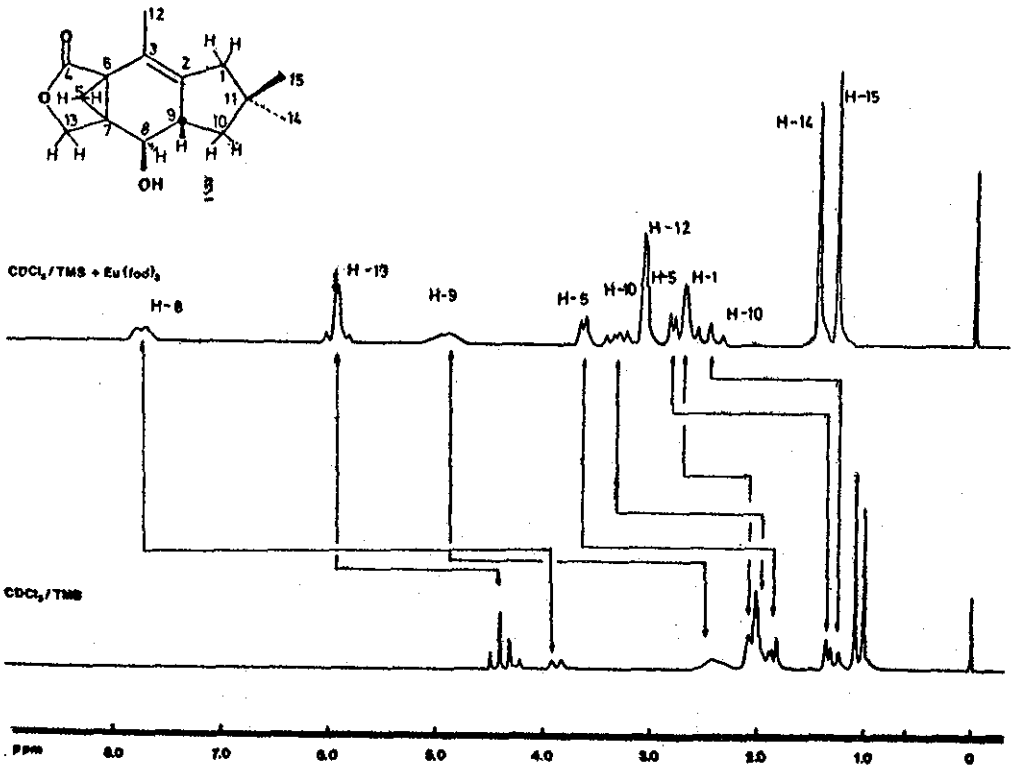
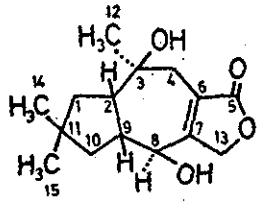


Fig. 1

However, all these data discussed above do not permit to draw far going conclusions concerning the geometry of isolactarorufin and the lactonic carbonyl position. We assume by analogy to all lactarane lactones that the carbonyl group has to be located on the same side as the methyl group (C-12). This assumption is supported by the observation that any chemical change involving the secondary alcohol group (at C-8) had a distinct influence on the signal of lactonic methylene protons. Further, in analogy to all lactaranes we accepted the cis-geometry of the cyclopentane/cyclohexane ring junction; and as it

was proved above the β-configuration of the secondary alcohol group. It follows from the examination of Dreiding models that the more favoured configuration of the tertiary alcohol group is beta.

The comparison of the observed and calculated $Eu(fod)_3$ induced chemical shifts revealed a very good consistency for all protons of dehydrated isolactarorufin with the cyclopropane β-methylene; hence the spatial structure of isolactarorufin (1) is as shown:



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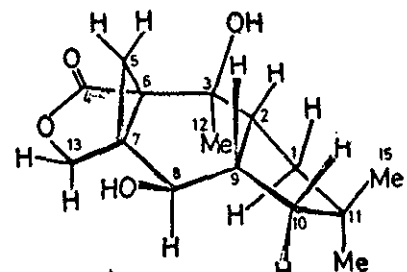
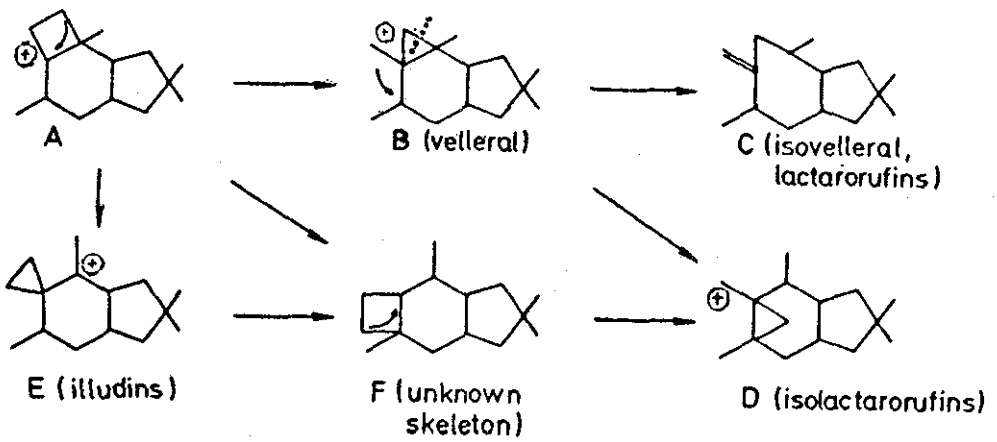


1

Fig. 2

The co-occurence of isolactarorufin with lactarorufins is a puzzling problem from the biogenetic point of view. The biogenetic pathway leading to lactarane skeleton with a cyclobutane intermediate A, common to most of polycyclic sesquiterpenes with a gem-dimethyl cyclopentane ring was suggested before (3,7). The cyclobutane ring contraction of A with the formation of B would lead to velleral (7) and the cyclopropane ring cleavage to isovelleral and lactaranes (C as an intermediate). One could also assume another cyclopropane ring cleavage (along the dotted line) with hydride shift from C-7 to C-4, followed by the reproduction of cyclopropane ring on C-6/C-7, giving rise to isolactarane skeleton. Another possiblity is represented by the sequence A → E → F → D, (or A → F → D), in

which the intermediate E leads to the formation of illudins (5). In this case a cyclobutane compound F should be the next intermediate, but to our knowledge none of the known sesquiterpenes has such a skeleton. Therefore we propose the sequence A⟶ B⟶ D as the possible biogenetic pathway for isolactarorufins (Scheme 2).



Scheme 2

Full report on isolactarorufin including absolute configuration determination will appear in the Polish journal Roczniki Chemii.

References

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Tetracyclic sesquiterpene lactone from European mushroom *Lacterius rufus*, the use of ^{13}C NMR and lanthanide shifted PMR spectra for structure determination, biosynthesis.

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