thought to be oxidized to 2-chloroacetate, which is dehalogenated to glycolate[13], a metabolite readily utilized as a carbon source by strain DE2 as well as many other bacteria.

*While the present paper is a short communication, the editors include it here as a useful appendage to the preceding review papers.

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Short Communications

The microbial oxygenation of the benzylisoquinoline alkaloid laudanosine[1]

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Summary. The microbial transformation of the benzylisoquinoline alkaloid laudanosine by a strain of *Pseudomonas putida* gives a metabolite in which O-demethylation of 1 methoxyl group of ring C, and introduction of 1 ketonic oxygen at C_9 and 1 phenolic oxygen at ring C have occurred. Also, O-methylcoripalline is formed in this transformation.

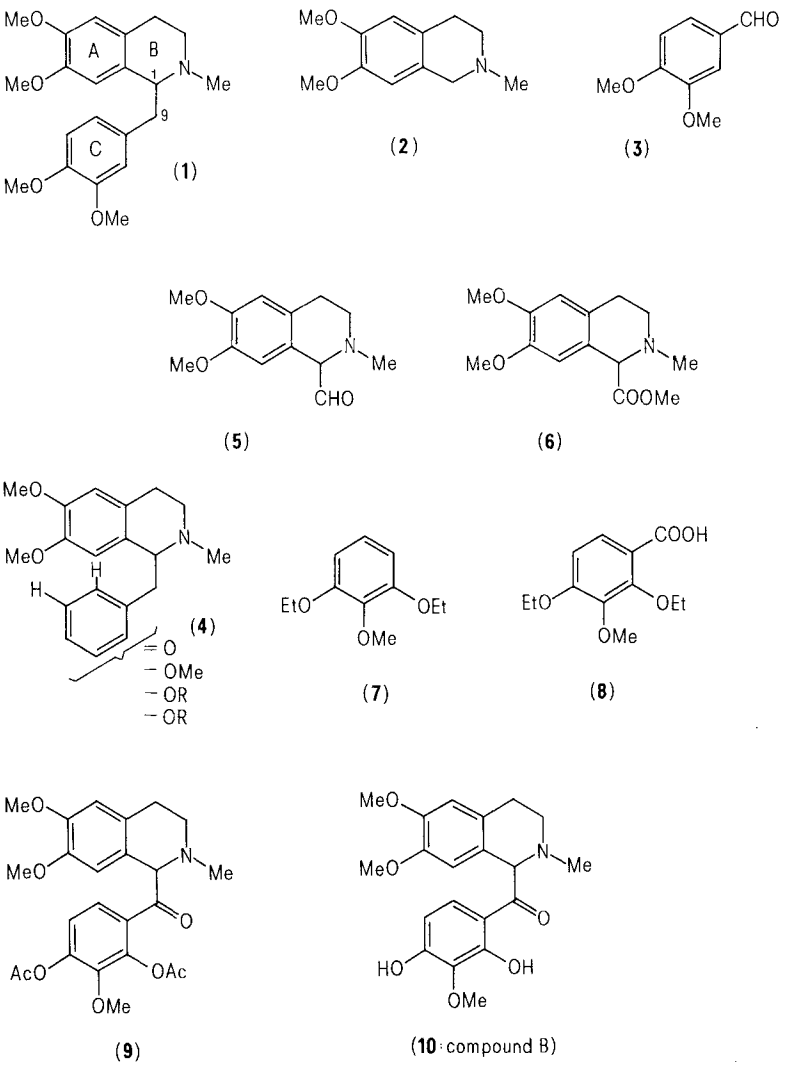
Laudanosine (**1**) is a minor opium alkaloid with the benzylisoquinoline skeleton. The chemical and biological importance of benzylisoquinoline alkaloids has stimulated many studies on the regiospecific oxygenation and the oxidative cyclization reaction[3,4]. The chemical oxidation of laudanosine with lead (IV) acetate or Fenton's reagent (Fe^{II}-H_2O_2) gives only O-methylcoripalline (**2**) and veratraldehyde (**3**), resulting from 'benzylic fission' between carbons 1 and 9. Neither cyclization to other alkaloids nor further functionalization of the benzylisoquinoline skeleton have been noted[5].

Here we report the results of the microbial transformation of laudanosine with a strain of *Pseudomonas putida,* isolated from a biological waste water treatment plant by an enrichment technique in the presence of laudanosine as the only carbon and energy source. This organism was grown on Raymond and Davis[6] liquid mineral salts medium with 0.5‰ (w/v) laudanosine. Under these culture conditions the formation of a compound absorbing at 395 nm at pH = 7 was detected.

For transformation experiments the *Pseudomonas putida* cells were inoculated into 750 ml flasks containing 0.5‰ laudanosine in 150 ml mineral medium. The flasks were incubated with shaking at 30 °C for 96 h.

The metabolites were isolated as follows: extraction of the culture broth with n-butanol gave a residue which was chromatographed on silica gel (R = 100). 15% of O-methylcoripalline (**2**) (based on the initial amount of laudanosine) and the yellow metabolites A, 6%; B, 12% and C, 6% were isolated. In the mass-spectrum of B were apparent peaks at m/e = 373 (M^+), 354, 207 and 193. This suggested that compound B was derived from laudanosine by loss of 1 carbon atom and 4 hydrogen atoms and introduction of 2 oxygen atoms. One of these oxygen atoms was part of a carbonyl function, as shown by the band at 1695 cm^{-1} in the IR (nujol).

The NMR-spectrum of B in $CDCl_3$ showed the N-methyl hydrogens as a singlet at 3.40 δ and the hydrogens of 3 methoxyls as singlets at 3.75 δ (3 hydrogens) at 4.15 δ (6 hydrogens). This suggested that the 1 carbon loss resulted from cleavage of 1 methoxyl group. Furthermore, 4 aromatic hydrogens were present. Two of them were singlets at 6.42 δ and 7.72 δ and 2 were doublets centered at 7.10 δ and 7.65 δ with J = 7 cps, suggesting an ortho arrangement. Since laudanosine has 5 aromatic hydrogen atoms, these data suggested that the additional oxygen atom in B could be a nuclear hydroxyl. An indication of the structure of B came also from the observation that in its



mass-spectrum the fragment at m/e = 207 attributable to the O-methylcoripalline ion was present. This suggested that the isoquinoline part in B was the same as that in laudanosine.

Compounds A and C were not isolated in a pure state since they equilibrated with B by acid-catalyzed treatment or even by silica gel chromatography. Their IR-spectrum was very similar to that of compound B. In particular, a broad band at 1670 cm^{-1} was present ($CHCl_3$ solution). However, compounds A, B and C were transformed by treatment with acetic anhydride into the same diacetyl derivative. This had the molecular formula $C_{24}H_{27}NO_8$ and m.p. 130–131 °C. Its mass-spectrum showed peaks at m/e = 457 (M^+); 438 (M^+-18); 384 (M^+-42-31); 371 (M^+-2[43]); 275 (M^+-2[60]-2[31]); 250 (M^+-207); 207 (M^+-250). In particular, it was apparent that the diacetyl derivative was constituted of the isoquinoline moiety of O-methylcoripalline (fragment at m/e = 207) attached to a fragment of m/e = 250 bearing 1 carbonyl group, 1 methoxyl group, 2 acetoxyl groups and 2 ortho aromatic hydrogens on a 7 carbon atom structure (4: R = Ac).

In the IR-spectrum (nujol) of compound (**4**) bands at 1765 and 1685 cm^{-1} were present. They were attributable to the acetoxyl carbonyls and to an aromatic ketone respectively. In the NMR in $CDCl_3$ the hydrogens of the acetoxyl groups resonated at 2.15 δ and 2.40 δ. The former had a long range coupling of 1 cps with the hydrogens of the N-methyl group resonating at 3.80 δ. The aromatic hydrogen atoms resonated at 6.95 δ and 7.08 δ as doublets with J = 8 cps and 7.18 δ and 7.30 δ as doublets with J = 1.5 cps. Thus the aromatic carbonyl group was assigned to position 9.

Degradation experiments allowed assignment of the positions of the other functional groups. Permanganate oxidation of the diacetyl derivative followed by treatment with diazomethane gave a mixture of the aldehyde (**5**) and the ester (**6**), confirming the structural assignments shown in (**4**). Permanganate oxidation of the diethyl derivative (4: R = Et) gave the ether (**7**). An authentic specimen of this was obtained by ethylation of pyrogallol-2-methyl ether. The isolation of (**7**) allowed assignment of structure (**9**) to the diacetyl derivative. In fact, the acid (**8**) first formed by oxidation with permanganate is expected to lose carbon dioxide easily. Hence, to compound B has to be assigned the structure (**10**) and compounds A and C could be 2 of the possible tautomers of (**10**).

From the cultures we could not isolate products deriving from oxidation of the N-methyl group.

We conclude that the metabolites are derived from monodemethylation of laudanosine, aromatic hydroxylation and introduction of 1 carbonyl function in position 9. These reaction steps are likely to be either enzyme-catalyzed or derived from autooxidation processes. The O-demethyla-

tion has been noted in biological systems and has been reported to occur frequently in microbial transformations of several natural aromatic compounds[7]. Moreover, the main metabolic reaction of *Cuninghamella echinulata*, in the presence of laudanosine, has been reported to be O-demethylation in the benzylic portion of the molecule[8]. The nature of the 2 oxygenation steps and the sequence of these 3 transformation steps are at present under investigation.

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Petiodial, a new monocyclic diterpenoid from the Mediterranean green alga *Udotea petiolata*[1]

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Summary. The structure of a new monocyclic diterpenoid, petiodial (**4**), present in the green seaweed *U. petiolata*, has been determined on the basis of physico-chemical data. This alga also produces udoteal (**3**), a feeding deterrent metabolite previously isolated from the taxonomically related species *U. flabellum*.

In 1981 Nakatsu et al.[2] reported the presence in the Caribbean green alga *Udotea flabellum* of 2 monocyclic aldehydic diterpenes, udoteafuran (**1**) and udoteatrial (**2**); the latter, which seems to be responsible for the antimicrobial activity of the crude extract, was isolated as a cyclic diacetate after treatment with Ac_2O. More recently udoteal (**3**), a 1,4-diacetoxybutadiene-containing diterpene, biogenetically related to **1** and **2**, was found in the same material by Paul et al.[3]. Sesqui- and di-terpenes with this unusual functionality have been isolated from some other algae belonging to the families Caulerpaceae and Codiaceae (Siphonales)[4–7], and apparently function as chemical defence agents, since many of them have been proved to induce pronounced feeding avoidance in herbivorous fishes.

On pursuing our chemotaxonomically oriented studies on marine green algae[8,9], we have been investigating *Udotea petiolata*, a seaweed very common in the Mediterranean, and belonging to the same genus of *U. flabellum*. We wish to report here that this alga also produces udoteal (**3**), as well as a new related metabolite (**4**), that we named petiodial.

Material and methods. Samples of *U. petiolata* were collected in the bay of Naples, Italy, during the spring 1982. The dried powdered whole plants (29 g, after extraction) were extracted 3 times with $CHCl_3$ and the solvent was evaporated to afford a residue (2.4 g) which was partitioned by a Si gel column using as eluant increasing amounts of Et_2O in n-hexane. Fractions eluted with n-hexane-Et_2O (8:2) afforded mg 128 of udoteal. Fractions eluted with n-hexane-Et_2O (75:25) were rechromatographed on PLC using C_6H_6-AcOEt (7:3) as eluant. The band Rf 0.75 (UV-light), scraped and eluted with Et_2O, gave mg 39 of pure petiodial as a colorless oil.

Results and discussion. Udoteal was identified by comparison of its spectral (PMR, CMR, UV, IR and MS) data with those reported by Paul et al.[3].

Petiodial, $[\alpha]_D = -25.7$ (c = 0.5, in $CHCl_3$) has molecular formula $C_{22}H_{32}O_4$ (from HRMS on the parent ion).

The part structure C_9-C_{18} in **4** could be deduced by comparison of its PMR- and CMR-spectra, performed in $CDCl_3$ using a Bruker WH 270 spectrometer, with those of nerolidol[10] and udoteal [PMR: δ 5.10 (1H, bt, J = 7Hz, 10-H), 5.05 (1H, bt, J = 7Hz, 14-H), 1.67 (3H, bs, 16-H_3), 1.60 (3H, bs, 17-H_3) and 1.58 (3H, bs, 18-H_3). CMR: δ 134.8 (s, C_{11}), 131.3 (s, C_{15}), 124.0 (d, C_{14}), 123.2 (d, C_{10}), 39.5 (t, C_{12}), 29.4 (t, C_9), 27.0 (t, C_{13}), 25.8 (q, C_{16}), 17.5 (q, C_{17}) and 15.9 (q, C_{18})].

The IR-spectrum of **4** indicated the presence of 2 aldehydic groups, one of which is α,β-unsaturated ($\nu_{max}^{CCl_4}$ 2740, 1720 and 1670 cm^{-1}). These assignments were confirmed by the expected PMR bands at δ 10.11 (1H, s, 1-H) and 9.64 (1H, d, J = 2Hz, 19-H) and by off resonance CMR signals at δ 204.3 (d, C_{19}), 187.6 (d, C_1), 158.4 (s, C_3) and 140.6 (s, C_2).

The presence of the CH_3COOCH_2-group linked to a fully substituted olefinic carbon was deduced from IR ($\nu_{max}^{CCl_4}$ 1740, 1235 cm^{-1}) and PMR [δ 5.06 (2H, s, 20-H_2) and 2.10 (3H, s, Me-CO-)] spectra.

Consideration of the molecular formula and overall unsaturations delineated from the CMR-spectrum which presents, in addition to the above mentioned bands and those of the acetoxyl group, only sp^3 carbon signals [δ 59.5 (t), 52.6 (d), 45.2 (d), 34.9 (t), 28.9 (t) and 26.4 (t)] showed petiodial to be a monocyclic diterpenoid.

We were able to determine the complete structure of petiodial from a detailed analysis of PMR-spectrum which contains further signals at δ 3.41 (1H, m, 6-H), 2.90 (1H, m, 7-H), 2.60 (2H, t, J = 4Hz, 4-H_2) and 1.82 (2H, m, 8-H_2). By irradiation at δ 2.90, the aldehydic signal at δ 9.64 collapsed into a singlet and the multiplets at δ 3.41 and 1.82 were simplified. On the other hand, the latter multiplet is also simplified by irradiation at δ 2.00 (9-H_2 frequency). Finally irradiation at δ 1.95 (tentatively the frequency of 5-H_2) caused the triplet at δ 2.60 to collapse into a singlet and simplified the multiplet at 3.41.

What remained for the final assignment of petiodial was the location of the aldehydic and CH_3COOCH_2-groups which could be linked to C_2 and C_3 respectively or vice