tions for the preferential formation of the above metabolites, estimating the yields by [4-^{14}C]-progesterone incubations.

Bioconversion of progesterone at a lower temperature (70 °C; experiment 2, table) resulted in the recovery of pregn-4-en-20α-ol-3-one (**4**) as the sole product, although the overall conversion was lower (4%) than at 85 °C (14%).

When at 70 °C the oxygen availability was increased by agitation of the incubation flask, only pregn-4-en-6α-ol-3,20-dione (**5**) and pregn-4-en-6β-ol-3,20-dione (**6**) were recovered in about the same amount (experiment 3, table), the total yield being 15%.

As previous work on this microorganism showed that several organic solvents have permeabilizing effects on the cell membrane without causing any significant lysis[7], we performed incubation experiments (experiment 4, table) at 85 °C with acetonized[7] resting cells. In these conditions a strong increase of the total conversion (24%) of progesterone was observed, while the metabolite pattern resembled that of experiment 1, the only difference being the lack of **4** and the relative increase of pregn-4-en-3,6-20-trione (**2**) and 5α-pregnan-3,6,20-trione (**3**).

To evaluate the usefulness of acrylamide trapped cells[7] in this process, progesterone (**1**) was incubated in the conditions of experiment 1, using an amount of acrylamide beads (300 g) containing about 70 g of wet cells. The results of this experiment (experiment 5, table) were about the same as in experiment 4, in which acetonized resting cells were used, and are in accordance with the conjecture[7] that polymerization conditions cause a permeabilization of the cellular membrane similar to that obtained by solvent treatment.

In conclusion the results of the above experiments suggest that at the lower temperature (70 °C) the hydroxylative and oxidative systems are inactive; however when the cells are shaken at 70 °C the increased oxygen availability gives rise only to the hydroxylated metabolites. The permeabilization of cell membranes results in a generalized increase of progesterone conversion.

Further work is in progress to optimize the bioconversion yield and to select the conditions better for obtaining particular metabolites.

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The structure of ceroplastodiol, a new tricyclic sesterterpene isolated from insect wax[1]

L. Quijano, J.S. Calderón and T. Ríos

Instituto de Química de la Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, México 20, D.F. (Mexico), 21 April 1980

Summary. The isolation and structure elucidation of cereoplastodiol, a new tricyclic sesterterpene isolated from wax of the insect *Ceroplastes albolineatus* are reported.

In continuation of our search for new sesterterpenic compounds in the wax of the scale insect *Ceroplastes albolineatus* Cockerell (Coccidae)[2], which has been previously shown to be a source of different types of compounds formed from 5 isoprene units (sesterterpenes), we have undertaken the study of a new collection of the insect's wax and succeeded in isolating a new tricyclic sesterterpene, which we have named ceroplastodiol (**1a**), and its corresponding diacetate (**1b**) and a mixture of the monoacetates (**1c**) (**1d**).

Ceroplastodiol (**1a**), m.p. 120–2°, $[\alpha]_D + 143.7(CHCl_3)$ has the composition $C_{25}H_{40}O_2$ and its MS displayed a molecular ion peak at m/Z 372 and a base peak at m/Z 354 ($M^+ - H_2O$) along with peaks at m/Z 336 ($M^+ - 2H_2O$), 227 ($M^+ - H_2O - C_8H_{15}O$). The IR-spectrum showed absorptions at 3450, 1650, 855, 840 cm^{-1} and UV-absorption at 213 nm (log ε, 3.038) indicating the presence of OH groups and double bonds.

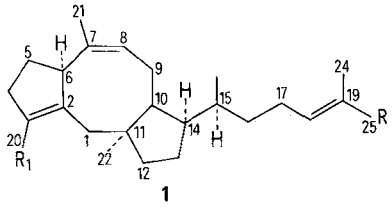
The PMR of ceroplastodiol (**1a**), revealed signals due to 2 vinyl methyl groups at δ 1.62 and 1.70, a secondary methyl group doublet at 0.80 ($J = 6.5$ Hz) and a tertiary methyl group singlet at 0.74. The last 2 methyl groups of the tricyclic skeleton, ceroplastane (**2**) have to be methylenes bearing hydroxy groups as indicated by the appeareance of 2 broad singlets at 4.02 and 4.22 which shifted downfield (4.38 and 4.57) upon acetylation. The PMR-spectrum also showed signals due to vinyl protons as triplets at δ 5.42 and 5.55 ($J = 7.0$ Hz), a broad triplet at 3.98 ($J = 8.0$ Hz) was assigned to H–6 doubly allylic and a doublet at 2.48 ($J = 14.0$ Hz) to one of H–1 protons.

Acetylation of **1a** afforded the diacetate **1b**. Catalytic hydrogenation in $EtOH/HClO_4$ using PtO_2 as catalyst gave the tricyclic hydrocarbon **2**, M^+ 346 ($C_{25}H_{46}$), which was identical with that obtained from ceroplastol acetate (**3**) under the same conditions.

Evidence for the position of the OH groups was provided by Sarett's oxidation of ceroplastodiol (**1a**), which led to the dialdehyde (**1e**) and the monoaldehyde (**1f**).

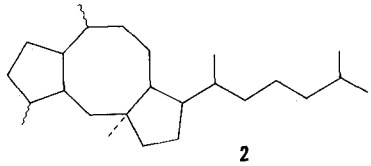
The IR-spectrum of **1e** contained bands at 2720, 1680, 1660 cm^{-1} and UV-absorption at 235 (log ε, 4.244), 260 nm (log ε, 4.184) indicating the presence of 2 different α, β-unsaturated systems. The PMR-spectrum showed 2 different aldehydic proton absorptions at 9.27 and 9.90. The downfield chemical shift at 6.36 of one of the vinyl proton signals as a triplet of quartets ($J = 7.0$ Hz, $J = 1.5$ Hz) indicated that one of the OH groups was at C–25 as in all the other sesterterpenes isolated from the wax, therefore the other one should be at C–20 since the vinyl proton broad triplet remained at 5.66. Furthermore the AB doublet due to one of H–1 protons was shifted downfield from 2.48 to 3.21 due to the anisotropic effect of the carbonyl group at C–20.

The most polar compound from the oxidation of ceroplastodiol (**1a**) was the monoaldehyde (**1f**). IR, νmax 2780,

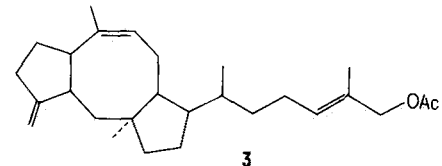


1

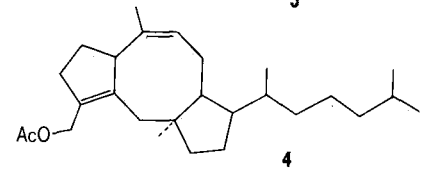
a $R=R_1=CH_2-OH$
b $R=R_1=CH_2-OAc$
c $R=CH_2-OAc$ $R_1=CH_2-OH$
d $R=CH_2-OH$ $R_1=CH_2-OAc$
e $R=R_1=CH=O$
f $R=CH_2-OH$ $R_1=CH=O$
g $R=CH_2-OAc$ $R_1=CH=O$
h $R=CO_2Me$ $R_1=CH_2-OH$



2



3



4

1670 cm^{-1}, UV, λmax 206 nm (log ε, 3.693) 258 nm (log ε, 3.937). Its PMR-spectrum indicated that the C–20 hydroxyl group was oxidated, since no shift of the vinyl protons was observed, but the AB doublet due to one of H–1 protons was shifted to 3.20. Acetylation of **1f** gave the acetate **1g**.

Catalytic hydrogenation of ceroplastodiol diacetate (**1b**) with PtO_2 in EtOH gave the product **4** in which the acetoxy group of the side chain was lost, as indicated by the PMR-spectrum which showed only 1 methyl acetate group at 1.99, 1 vinyl methyl group at 1.68 and a vinyl proton at 5.47. The MS exhibited a molecular ion peak at m/Z 400 and peaks at m/z 340 (M^+-AcOH) and 227 ($M^+-AcOH-C_8H_{17}$).

All the above results are in good agreement with the proposed structure of ceroplastodiol (**1a**). Final confirmation of the structure **1a** was achieved by treatment of methyl ceroplastolate[3] (**1h**) with $LiAlH_4$ to give ceroplastodiol (**1a**).

1 Contribution No. 550 from Instituto de Química de la Universidad Nacional Autónoma de México.
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A simple synthesis of (E)-3-formylbut-2-enenitrile[1], and its use as a precursor of isotope-labelled zeatin and (±)dihydrozeatin[2]*

Shi-chow Chen

Special Projects and Microbiology Department, Alberta Research Council, Edmonton (Alberta, Canada T6G 2C2), 4 November 1980

Summary. (E)-3-Formylbut-2-enenitrile (**4**) is synthesized in 2 steps by reacting pyruvaldehyde dimethylacetal and acetonitrile in the presence of sodium methoxide, followed by acid hydrolysis to give 58% overall yield on distillation. The aldehyde **4** can be stepwise and selectively reduced to (E)-3-hydroxymethylbut-2-enylamine (**7a**) in 37% total yield or exhaustively reduced in 1 step to (±)-4-hydroxy-3-methylbutylamine (**6**) in 46% total yield. Compound **7a** and **6** can be condensed with 6-chloropurine to give zeatin and (±)dihydrozeatin respectively. This provides a readily accessible method for isotope-labelled zeatin and its derivatives at side chain.

Zeatin (**1**) and dihydrozeatin (**2**) are highly active naturally occurring plant hormones which induce cell divisions in tissue culture[3]. Synthesis of zeatin[4–11] is of continuing interest to both organic and agricultural chemists both because of the difficult problem in the construction of the small, but highly functionalized, key intermediate **7a** and also because of the potential importance of the plant hormone in agricultural and biological research.

Previous syntheses of **7a** involve many steps, provide low yields and require the difficult separation of the geometric isomers. This latter problem has been diminished by a method reported by Ohsugi et al.[4]. We now report a novel and efficient synthesis of (E)-3-formylbut-2-enenitrile (**4**), which can be selectively or exhaustively reduced to **7a** or **6**. Compounds **7a** and **6** can be condensed with 6-chloropurine to give zeatin[5] and (±)dihydrozeatin[12,13] respectively.

Our method not only compares with that of Ohsugi et al.[4] in the overall yield, but also provides a versatile process for the syntheses of isotope-labelled zeatin and its derivatives. We believe that the regiospecific synthesis of the highly functionalized isoprenoid compound **4** and the underlying reactions may also have wider synthetic implications.

We have found that whereas a methyl ketone such as acetophenone cannot be satisfactorily condensed with acetonitrile[14,15], an acetal of pyruvaldehyde, in which the α,α-dialkoxy substituent has a stabilizing effect on the polarizing carbonyl group, can be condensed with acetonitrile with surprisingly good results (65–75% isolated yield).

Thus, pyruvaldehyde dimethylacetal is condensed with a large excess of acetonitrile in the presence of a strong base (1 mole equiv of $NaOCH_3$, reflux under nitrogen for 8 h) to give an isomeric mixture of the corresponding acetal of 3-