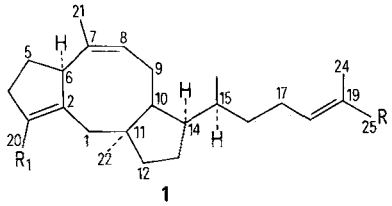
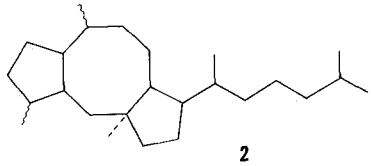


1

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 $R=CO_2Me$ $R_1=CH_2-OH$



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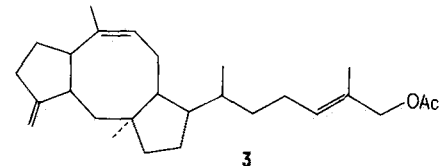

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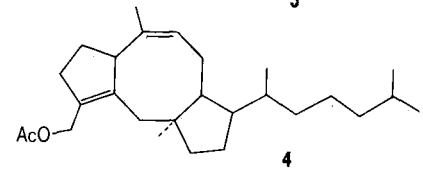

4

1670 $cm^{-1}$, UV, $\lambda$max 206 nm (log $\varepsilon$, 3.693) 258 nm (log $\varepsilon$, 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.
2 L. Quijano, J.S. Calderón and Tirso Ríos, Chem. Lett. *1979*, 1387 and references therein.
3 L. Quijano, J.S. Calderón and Tirso Ríos, Chem. Ind. *1979*, 592.

## A simple synthesis of (E)-3-formylbut-2-enenitrile[1], and its use as a precursor of isotope-labelled zeatin and (±)dihydrozeatin[2]*

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*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.

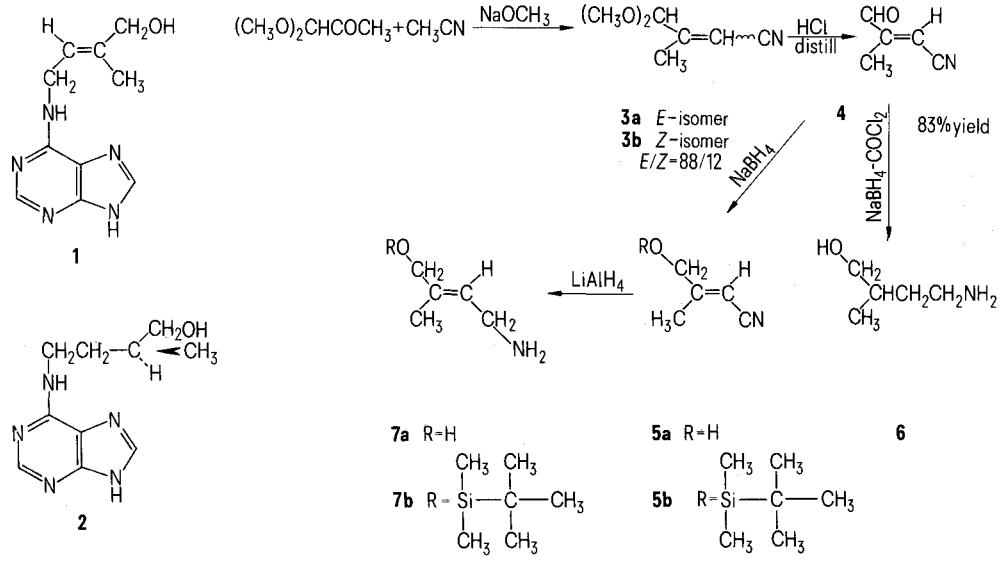
Zeatin (**1**) and dihydrozeatin (**2**) are highly active naturally occurring plant hormones which induce cell divisions in tissue culture[3]. Synthesis of zeatin[4–11] is of continuing interest to both organic and agricultural chemists both because of the difficult problem in the construction of the 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 $\alpha,\alpha$-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-




formylbut-2-enenitrile **3** (b.p. 32–41°/0.2 mm, 70% isolated yield) with predominantly E-configuration (E/Z = 88/12).

Hydrolysis of above isomeric acetal mixture **3** (0.5 N methanolic HCl solution) and distillation yielded exclusively the E-isomer of the $\alpha,\beta$-unsaturated aldehyde (**4**) in a pure state (83% yield), eliminating the need for separating geometric isomers at any point in the overall synthetic pathway. Presumably, the corresponding Z-isomer, further reacts intra- or intermolecularly to form a higher boiling fraction[16,17]. Compound **4** has the following physical parameters: b.p. 71°/11 mm; NMR (60 Hz, $CDCl_3$) $\delta$9.73 (s, 1H), 6.40 (q, J = 1.5 Hz, 1H), 2.10 (d, J = 1.5 Hz, 3H); IR (film) 2730 ($\nu$CH of CHO group), 2220 ($\nu$C≡N) and 1705 $cm^{-1}$ ($\nu$C=O of $\alpha,\beta$-unsaturated aldehyde); 2,4-DNPH, m.p. 276 °C. The liquid aldehyde and the hydrazone gave correct elemental analysis and mass spectral data ($M^+ = 95$ and 275 respectively).

Attempts to convert aldehyde **4** to the required unsaturated amino-alcohol **7a** in 1 step by means of selective hydride reduction or catalytic hydrogenation were not successful. However, the aldehyde **4** was selectively reduced with sodium borohydride (1 mole equiv, 15 min) in the presence of methanol to give cyanoalcohol **5a**[5,19] in excellent yield (~95%) without contamination of a saturated alcohol. Alternatively, compound **4** can be exhaustively reduced with a metal hydride-transition metal system[20] such as $NaBH_4$-$COCl_2 \cdot 6H_2O$ in the presence of methanol to give (±)-4-hydroxy-3-methylbutylamine (**6**)[13,18] in good yield (80%).

The cyanoalcohol **5a** obtained above was transformed to *trans*-3-hydroxymethylbut-2-enylamine (**7a**) via protection of the allylic hydroxy function as the t-butyldimethylsilyl ether[21] **5b** (b.p. 72–74°C/0.05 mm) followed by reduction with lithium aluminum hydride (ether) to give the corresponding amine **7b** (70% yield). Removal of the silyl group with dilute methanolic sulfuric acid solution gave aminoalcohol **7a** in quantitative yield as the sulfate salt[9].

Utilization of sodium tetradeuteridoborate or lithium tetradeuteridoaluminate for the synthesis of **7a**, gave a mono- or a dideuterated derivative of **7a** with the label(s) at the corresponding allylic methylene (–O–CHD– or =N–$CD_2$–) positions. Similarly, $^{13}C$ or radioisotope $^{14}C$ or $^{3}H$ can be incorporated into the zeatin side chain, isotope substitution of which would not be readily accessible by alternative methods.

From the synthetic point of view, several noteworthy features follow: a) in the presence of a strong base, acetonitrile can be condensed to an activated methyl ketone and then dehydrated to give an $\alpha,\beta$-unsaturated nitrile; b) a variety of isoprenoid synthetic intermediates having a basic functional array of type **4** may be readily prepared; and c) zeatin and dihydrozeatin isotope-labelled at the side chain can be synthesized in good yield, and may prove to be very useful compounds to provide new entry into the biological study of cytokinins[3].

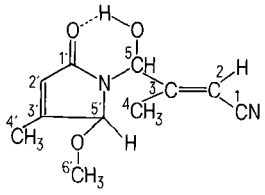
* Contribution No. 1064, Alberta Research Council.

1 'Process for the Preparation of *trans*-3-formylbut-2-enenitrile' S. Chen and J. MacTaggart, patent application registered in Canada and the United States of America.

2 Acknowledgment. The author thanks Mr J.M. MacTaggart and Mrs C.M. Goulet for valuable experimental assistance and Dr R.M. Elofson for helpful discussions.

3 For recent review cf: D.S. Letham, in: Phytohormones and Related Compounds: A Comprehensive Treatise, ed. D.S. Letham, P.B. Goodwin and T.J.V. Higgins, vol. 1, Cytokinins, p. 205, Elsevier North-Holland, Amsterdam 1978.
4 M. Ohsugi, I. Ichimoto and H. Ueda, Agr. Biol. Chem. *38*, 1925 (1974).
5 D.S. Letham, R.E. Mitchell, T. Cebalo and D.W. Stanton, Aust. J. Chem. *22*, 205 (1969).
6 G. Desvages and M. Olomuki, Bull. Soc. chim. Fr. *1969*, 3229.
7 D.S. Letham and H. Young, Phytochemistry *10*, 2077 (1971).
8 J. Corse and J. Kuhnle, Synthesis *1972*, 618.
9 M. Ohsugi, S. Takahashi, I. Ichimoto, and H. Ueda, Nippon Kogeikagaku Kaishi *47*, 807 (1973).
10 G. Shaw, B.M. Smallwood and D.V. Wilson, J. chem. Soc. *C, 1966*, 921.
11 R. Mornet and L. Gouin, Tetrahedron Lett. *1977*, 167.
12 K. Koshimizu, T. Kusaki, T. Mitsui and S. Matsubara, Tetrahedron Lett. *1967*, 1317.
13 T. Fujii and N. Ogawa, Tetrahedron Lett. *1972*, 3075.
14 R.S. DeSimone, U.S. Patent No. 3, *960*, 923 (1976).
15 S.A. DiBiase and G.W. Gokel, Synthesis *1977*, 629.
16 Fine white needles, m.p. 156–157 °C, are isolated from the pot residue after distillation. This compound, $C_{11}H_{14}O_3N_2$, is assigned to the structure shown below on the basis of the spectral data: UV $\lambda_{max}$ 211 nm ($\varepsilon$ 17,000) in ethanol; MS (70 eV) m/e (rel. int.) 222 (24), 191 (13), 156 (34), 125 (55), 110 (56) and 97 (100); IR (KBr) 3250 ($\nu$OH), 2215 ($\nu$C≡N), 1675 and 1643 (C=C–CO–N) and 1090 $cm^{-1}$ ($\nu$C–O–C); $^1$H-NMR (60 MHz, in DMSO-$d_6$) $\delta \sim 1.95$ (2 superimposed d,

J = ~ 1 Hz, 2 $-CH_3$), 3.26 (s, 3H, $-OCH_3$), 5.23 (d, J = 9 Hz, 1H, $-CH(OH)-$), 5.56 (m, 1H, $-(CH_3O)CH-N=$), 5.76 (m, 1H, =CH−), 5.93 (m, 1H, =CH−), and 6.25 (d, J = 9 Hz, 1H, $D_2O$ exch.); $^{13}$C-NMR (20.1 MHz, in DMSO-$d_6$, ppm) 171.0 (C-1′), 161.5 and 160.8 (C-3 and C-3′), 120.2 (C-2′), 117.3 (C-1), 96.1 (C-2), 81.0 (C-5, C-5′), 55.6 (C-6′), 17.6 (C-4) and 13.3 (C-4′) [see Chen et al.[19] for the $^{13}$C-NMR of structure **5a**].



17 The possibility that the acetal **3b** undergoes acid catalized isomerization to the thermodynamically more stable **3a** prior to hydrolysis has been ruled out by an independent experiment, in which hydrolysis of Z-isomer of pyruvaldehyde diisopropylacetal did not give aldehyde **4**.
18 Attempt to convert the unsaturated aldehyde **4** by baker's yeast fermentation (F.G. Fisher and O. Wiedermann, Annalen *513*, 265 (1934)) to (3S)-4-hydroxy-3-methylbutyronitrile, which has a chiral carbon corresponding to the natural dihydrozeatin, gave the unsaturated alcohol **5a** instead.
19 S.C. Chen, R.M. Elofson and J.M. MacTaggart, J. Agric. Food Chem. *27*, 435 (1979).
20 T. Satoh and S. Suzuki, Tetrahedron Lett. *1969*, 4555.
21 E.J. Corey and A. Venkateswarlu, J. Am. chem. Soc. *94*, 6190 (1972).

## Effect of glucagon on ethanol oxidation in isolated rat liver cells[1]

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*Summary.* Addition of glucagon 5 min after ethanol was found to stimulate the rate of ethanol oxidation in hepatocytes isolated from starved rats. This stimulation is of the same order of magnitude as that mediated by asparagine. The glucagon effect is suppressed by antiproteolytic agents such as insulin or $NH_4Cl$. The stimulating effect of glucagon on ethanol oxidation is probably linked to enhanced proteolysis and an elevated glutamate level in the hepatocytes.

In fasting pigs[2], in rats[3] and in man[4], acute ethanol ingestion has been shown to elevate the blood glucagon level. Conversely, glucagon administration has been observed to result, under defined experimental conditions, in a significantly increased rate of alcohol metabolism[5,6].

Thus, the existence of a relationship between glucagon concentration and ethanol oxidation rate is conceivable. Freshly isolated hepatocytes responsive to hormone stimuli seem to be a useful model to investigate such a connection at the cellular level.

This paper presents the action of glucagon on ethanol oxidation in liver cells isolated from starved rats and incubated either without energetic substrates other than ethanol or in the presence of asparagine, an amino acid which by itself favours ethanol oxidation[7]. For purpose of comparison, the action of insulin and of cortisol have also been investigated.

*Materials and methods.* Isolated hepatocytes were prepared as previously described[7] from overnight fasted female Wistar rats (180–200 g b.wt), the viability of the hepatocytes being evaluated according to Beaugé et al.[8]. Suspensions of hepatocytes (equivalent to 50 mg liver wet wt/ml) were incubated at 37 °C in Krebs-Ringer bicarbonate buffer pH 7.4 gassed with 95% $O_2$–5% $CO_2$. Cortisol 21-phosphate (10 μM) was added 60 min before ethanol (8 mM), $NH_4Cl$ (4 mM) and asparagine (4 mM) 5 min before ethanol, whereas glucagon (0.1 μM) and insulin (0.1 μM) were added 5 min after ethanol. 40 min after ethanol addition, perchloric acid deproteinization was carried out. Ethanol, glucose, urea, aspartate and glutamate were assayed enzymatically in the supernatant according to Bergmeyer and Gawehn[9].

Cortisol 21-phosphate, glucagon and insulin were purchased from Serva (Heidelberg, Germany), L-asparagine from Sigma (Saint-Louis, Mo, USA), enzymes and cofactors from Boehringer (Mannheim, Germany). The purity of

Effect of glucagon insulin, $NH_4Cl$ and asparagine on the rates of ethanol oxidation and on metabolite accumulation by isolated liver cells from starved rats

| Additions | Glucagon | Ethanol oxidation | Glucose | Urea | Aspartate | Glutamate |
|---|---|---|---|---|---|---|
| None | − | 0.75 ± 0.10 (6) | < 1 (6) | 11.4 ± 1.4 (6) | < 0.2 (6) | 1.3 ± 0.3 (6) |
| None | + | 1.66 ± 0.22 (6)[a] | 1.08 ± 0.22 (6) | 18.6 ± 2.2 (6)[a] | 1.60 ± 0.25 (6) | 2.2 ± 0.6 (6)[a] |
| Insulin | − | 0.86 ± 0.08 (3) | < 1 (3) | 11.9 ± 2.8 (3) | < 0.2 (3) | 1.5 ± 0.3 (3) |
| Insulin | + | 0.93 ± 0.09 (3) | < 1 (3) | 9.1 ± 2.4 (3) | < 0.2 (3) | 1.2 ± 0.2 (3) |
| $NH_4Cl$ | − | 0.92 ± 0.20 (3) | < 1 (3) | 47.4 ± 3.5 (3)[a] | < 0.2 (3) | 0.8 ± 0.2 (3) |
| $NH_4Cl$ | + | 0.70 ± 0.13 (3) | < 1 (3) | 44.9 ± 2.7 (3)[a] | < 0.2 (3) | 0.9 ± 0.2 (3) |
| Asparagine | − | 1.70 ± 0.10 (6)[a] | 13.6 ± 0.9 (6) | 36.8 ± 1.9 (6)[a] | 10.7 ± 1.6 (6) | 2.90 ± 0.25 (6)[a] |
| Asparagine | + | 1.84 ± 0.10 (6)[a] | 15.6 ± 0.5 (6)[b] | 47.5 ± 10.0 (5)[a,b] | 10.1 ± 2.0 (6) | 2.78 ± 0.40 (6)[a] |
| Insuline + asparagine | − | 1.95 ± 0.24 (3) | 12.4 ± 1.8 (3) | 40.6 ± 10.2 (3)[a] | 12.8 ± 0.9 (3) | 2.4 ± 0.4 (3)[a] |

Glucagon (0.1 μM) and/or insulin (0.1 μM) were added 5 min after ethanol (8 mM) whereas $NH_4Cl$ (4 mM) or asparagine (4 mM) were added 5 min before ethanol. The substrate determinations were performed 40 min after ethanol addition. The rates of ethanol oxidation are expressed as μmoles/min per g wet wt and the other substrates as μmoles/g wet wt. Each value is the mean ± SEM of the number of experiments with different cell preparations indicated in parentheses; within each experiment 3 determinations were performed.

Statistical significance: [a] $p < 0.01$ compared to results obtained without any addition except ethanol; [b] $p < 0.01$ compared to results obtained with the same additions except glucagon.