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δ-AMINO α-ACETYLENIC EPOXIDES.

Preparation and biological activity due to an aldehyde reductase inhibition

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Abstract

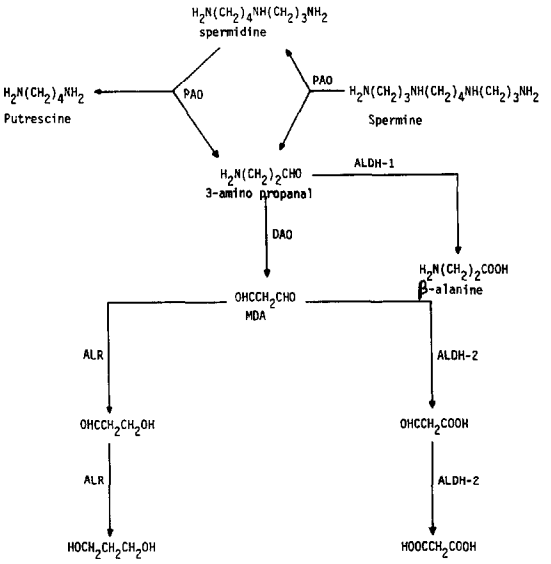
The preparation of several of the entitled epoxides 3 is described in two steps starting from 2-methyl 2-amino but 3-yne, the first one being the reaction of the lithium acetylide with a α-chloroaldehyde or ketone. In certain cases, this reaction is highly diastereoselective giving 96% of the R*S* diastereoisomer.

One of these epoxides (3a) inhibits very selectively the growth of transformed cells. This activity is due to the inhibition of aldehyde reductase, an enzyme involved in the intracellular transformation of malonaldehyde derived from polyamines into 1,3-propanediol.

It is now well established that the natural polyamines (putrescine, spermine and spermidine) play an important role in the regulation of cell growth (1) and that the intracellular concentration of these compounds is increased during the early stage of the transformation of cells by either oncogeneous viruses or chemical carcinogens (2). More recently, it was established that a part of the intracellular malondialdehyde (MDA) comes from the oxidation of spermine by the combined action of polyamine oxidase (PAO) and diamine oxidase (DAO) via 3-aminopropanal as intermediate (3). Consequently, it was proposed that the concentration of MDA in cells is regulated by four enzymes : PAO which transforms polyamines into 3-amino propanal and DAO which oxidizes this last compound are responsible for the increase of the MDA content while aldehyde dehydrogenases (AlDH) 1 or 2 and aldehyde reductase (AlR) transform it to malonic acid or 1,3-propanediol respectively ; 3-aminopropanal itself can be oxidized by AlDH 1 to the corresponding acid, β alanine, thus decreasing the MDA content (4) (scheme 1).

As it is well recognized that MDA, by its action upon DNA replication, is a powerful inhibitor of cell growth (5), we became interested in developing compounds able to decrease or suppress the activity of one of these enzymes, AlDH1 and 2 or AlR, involved in controlling intracellular MDA levels. It was postulated on starting this program that the activity of such inhibitors should be greater for transformed cells, and if so, this approach should allow us to accede to selective inhibitors with an evident interest in anti-cancer applications.

Our first results in this field showed that it is possible to have this kind of effect with certain α,α'-difunctionnal acetylenic compounds and mainly with 4-aminoacetylenic carbonyl compounds 1, the most effective in activity and selectivity being 4-amino 4-methyl 2-pentyne 1-al 1a (AMPAL) which proved to be an irreversible inhibitor of AlDH1. This compound selectively decreased the growth of transformed cells in in-vitro assays (6), and also exhibited an in-vivo antitumour activity towards certain leukemias and carcinomas (7).



PAO : Polyamine-Oxydase
DAO : Diamine-Oxydase
ALR : Aldéhyde-Réductase
ALDH-1 : Aldéhyde-deshydrogénase-1
ALDH-2 : Aldéhyde-deshydrogénase-2

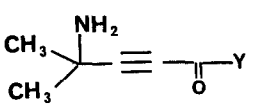
Scheme 1

Evidently, the main disadvantage of AMPAL 1a lies in its instability at room temperature, even in dilute aqueous solutions. For this reason, the stored diethyl ketal 2 has to be extemporaneously cleaved by treatment 12 hours at 37° by an equimolar amount of 0.2 M aqueous hydrochloric acid.

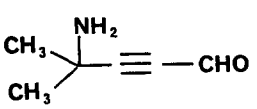
One possible explanation for the biological activity of AMPAL lies in a Michael addition of a nucleophilic site of the enzyme to the activated acetylenic bond. In our search for more stable analogs, we considered the possibility that α-acetylenic epoxides of general formulae 3 may have a comparable effect.

These compounds can either act by themselves as Michael acceptors or can isomerize to α -allenic ketones (8), compounds known (9) to be good inhibitors of certain enzymes by such a process.

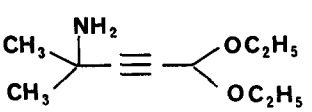
In this paper, we will describe the preparation of several of these epoxides 3 and will give the results concerning their activity on the growth of normal and transformed cells as well as their inhibitory action on aldehyde reductase.



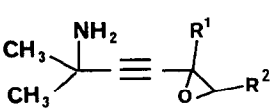
1



1a



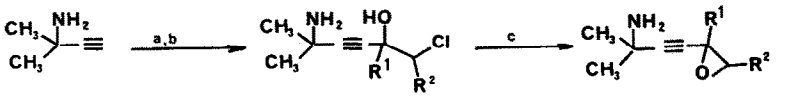
2



3

Preparation of the epoxides 3

These compounds have been prepared in two steps starting from the commercially available 2-methyl 2-amino but-3-yne 4 (scheme 2).



4 6 3

a) nBuLi, THF b) R^1-CO-CHCl-R^2 5 c) tBuOK/Et_2O

Scheme 2

The reaction of one molar equivalent of n-butyl lithium with 4 in tetrahydrofuran, followed by the addition of the α-chloroaldehyde or ketone 5 leads to the amino-chlorhydrin 6, smoothly cyclized to the epoxides 3 by treatment with potassium t-butoxide in ether (table 1).

The yield of the first step is uniformly good while the result of the cyclization depends on the nature of R^1 and R^2 which greatly influences the stability of the epoxide 3.

The best method for the purification of these compounds is distillation which, in the case of the very unstable 3d, is accompanied by an intense polymerisation while chromatography of the same substrate on diverse supports gives rise to a considerable loss of product. The other epoxides 3a-c are stable compounds which were stored several months in ethereal solutions at -20°C without noticeable alteration.

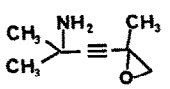
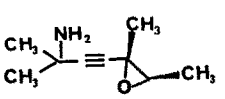
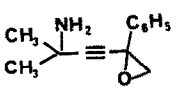
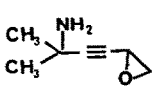
R^1	R^2	amino-epoxides 3	yield* of 6	yield of 3
Me	H	 3a	85%	78%
Me	Me	 3b	93%	45%
Ph	H	 3c	80%	45%
H	H	 3d	94%	5%

Table 1

* referred to α-chloro aldehyde or ketone 5

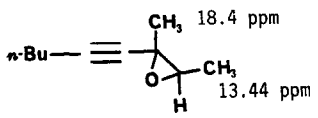
The diastereoselection observed in the case of 3b is noteworthy since the obtained epoxide is mainly under the configuration having the two methyl groups in cis position (for structural determination, see below) ; the trans isomer represents only 4% of the mixture as revealed by

capillary G.C. and confirmed by NMR. This result proved that the reaction of the lithium acetylide with 3-chlorobutan-2-one was highly diastereoselective giving predominantly the R*S* isomer of the intermediate chlorhydrin 6b.

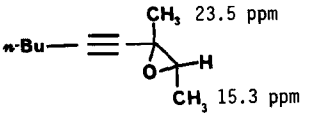
Such a diastereoselectivity in the reaction of organometallics with α-chloroketones has been previously reported but to a less extent, a diastereoisomeric ratio of 4 being generally observed (10), the more abundant isomer corresponding to the Felkin-Anh model (11).

The reaction of the lithium acetylide of 4 with 3-chloro butan-2-one being particularly diastereoselective, we studied the same reaction starting with other acetylides in order to test the generality of this selectivity. As seen in table 2, the results differ to a large extent from one case to the other : the reaction starting either from hexyne or phenylacetylene proved to be as diastereoselective as in the case of the amine 4 while this selectivity is lower when the sequence is run starting from the propargylic alcohol 2-methyl but-3-yne 2-ol or when the acetylide of 1-hexyne is condensed with 2-chloro butanal. Even if the reasons for such differences are unclear, it appears that this reaction of lithium acetylides with α-chloro carbonyl compounds can often proceed with a high degree of stereoselectivity (12).

As already mentioned, the structure of the main isomer of the epoxide in each reaction can be hypothetized as being R*S* on the basis of the Felkin-Anh model. This point was verified by ^{13}C NMR spectroscopy on the mixture 96/4 of both isomer of epoxide 7 resulting from the reaction of the butyne acetylide with 3-chloro butan-2 one. This spectrum shows a neat γ-shielding effect of the two epoxidic methyl groups in the predominant isomer referred to the minor one (13).



7(main isomer)



7(minor isomer)

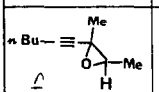
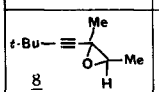
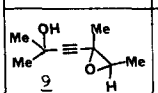
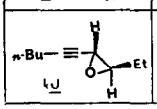
Epoxide (main isomer)	yield chlorhydrin	yield epoxide	cis/trans	diastereo-selectivity
3b	93%	45%	96/4	24
 7	96%	68%	96/4	24
 8	97%	76%	95/5	19
 9	73%	70%	85/15	5.7
 10	95%	57%	20/80	4

Table 2

Biological tests of the epoxides 3

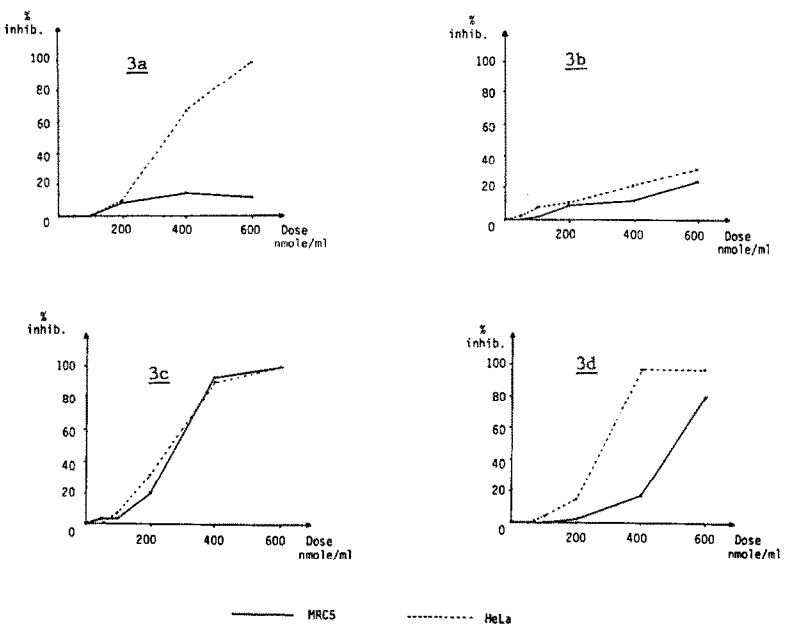
Inhibition of cell growth

The inhibitory action of the four epoxides 3a-d was firstly determined on the growth in culture of two kinds of cells : normal human embryonic lung fibroblasts MRC5 and a malignant cell line HeLa with concentrations of the epoxide in the culture medium up to 600 nmoles/ml (see experimental part for protocol). The results given in scheme 3 proved that the biological activity is fairly dependent on the substitution of the oxiranne : almost no activity is observed for 3b while 3c is a good inhibitor for both kinds of cells. A correct selectivity is exhibited by 3d at medium concentration (400 nmoles/ml) but this selectivity disappears for highter quantities of compound. The best of the epoxides 3 for both activity and selectivity is by far 3a, which has about the same characteristics as AMPAL 1a (6). Consequently, this compound was tested with other kinds of transformed cells (table 3) : in every case, an almost complete inhibition of cell growth was observed in the concentration range 400-600 nmoles/ml.

It is also noteworthy that the malondialdehyde content of HeLa cells treated by 600 nmoles/ml of 3a has increased to 30% compared to that in untreated cells, verifying that inhibition of growth is concomitant with the increase in the concentration of intracellular MDA.

Finally, the selective inhibition of the growth of transformed cells by this epoxide prompts us to determine whether the growth inhibitory effect would be more marked in the presence of AMPAL 1a which also selectively inhibits cell growth because of its activity on its target enzyme AlDH (6).

Accordingly, AMPAL and the epoxide 3a were added either individually or sequentually to HeLa cells in culture at 2.10^{-4} M, at which concentration they each have but a limited effect on cell growth (AMPAL 1a : 64% inhibition ; epoxide 3a : 21% inhibition). When added sequentially at the same concentration, an additive inhibitory effect of 93% was observed.



Scheme 3

Dose nmole/ml	MRC5	HeLa	HEp2	293
50	/	/	/	20
100	/	/	/	50
200	/	10	20	90
400	10	70	80	dead
600	10	90	dead	-

Table 3 : inhibition of cell growth by the epoxide 3a

Enzyme inhibition

The influence of the amino-epoxide 3a on the activities of aldehyde dehydrogenase and aldehyde reductase was studied : if AlDH is almost insensitive to 3a, AlR is completely inhibited in 30 mn at high concentrations (1000 nmoles/ml) while this activity is immediatly reduced to 10% at 800 nmoles/ml. If the contact between the enzyme and 3a is maintained during one hour, this activity is reduced to 25% by using only 200 nmoles/ml of the epoxide.

These results agree completely with the conjugated effect of 1a and AMPAL on the growth of cells since these two compounds both increase the intracellular content of malondialdehyde by inhibiting two different enzymes that regulate this content (see scheme 1).

In vivo tests

The in-vivo tests were performed on CBL black mice grafted with different kinds of tumours (L1210, P388, RBL5 and Lewis tumour) with the same experimental protocol used previously with AMPAL (7). Unfortunately, no antitumour activity was observed regardless of the type of tumour used (leukemia, carcinoma). This is probably due to the metabolism of the oxirane functionality by the epoxide hydrolase present in liver microsomes (14).

Conclusion

The results presented here show that the easily accessible and stable epoxide 3a is a selective inhibitor of the growth of certain transformed cells in culture. Unfortunately, this "in vitro" activity is not observed during "in vivo" tests on mice grafted with transplantable tumours due, at least in part, to the metabolism of this epoxide by hepatic epoxide hydrolase.

The main interest of this study is to provide some more evidence concerning the relationship between the inhibition of cell growth, the intracellular concentration of malondialdehyde and the activity of certain enzymes. Consequently, these results can help in providing specifications for the design of more effective inhibitors of the enzymes involved in the intracellular homeostasis of malondialdehyde.

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EXPERIMENTAL SECTION

- anhydrous THF is obtained by distillation over Na + benzophénone and anhydrous ether by distillation over $LiAlH_4$
- commercialy available organolithium are titrated before use by diphenylacetic acid (15)
- G.C analyses are performed on a GIRDEL-DELSI 330 chromatograph (flame ionisation detector) on 25m capillary columns : col.A (FFAP), col.B (OV1701). In the descriptions t_R=retention time ; θ = oven temperature

- IR spectra are recorded with a Perkin Elmer 298, neat or in solution in chloroform. Only the most prominent or diagnostic peaks are reported (cm^{-1}).
- ^{1}H NMR spectra are recorded on the following spectrometers ; Varian EM360 (60MHz) for routine spectra, Brucker 200WP, Brucker AM300 and Cameca 350, FT instruments operating at 200 and 350 MHz. ^{13}C NMR spectra were measured at 50.1MHz, 75 or 88MHz. Chemical shifts are expressed in ppm downfield from tetramethylsilane. Significant ^{1}H data are tabulated in the order : multiplicity (s,singlet ; d,doublet ; t, triplet ; q, quartet ; m, multiplet), coupling constant(s) in hertz, number of protons.
- Mass spectra (MS), m/z (relative intensity) are obtained from Varian-Mat CH5 ; VG30F ; NERMAG R1010S spectrometers. Hihg resolution determinations are done with an AEI-MS902. In all cases, the activation energy is 70eV.
- Acetylenic epoxides are oily compounds giving generally microanalysis results out of the commonly admitted limits, probably because of their relative unstability. However, their high resolution NMR spectra as well as their G.C. or T.L.C. analysis do not show the presence of other compounds ; their purity can be consequently estimated > 95%.
- 2-chloro butanal is prepared according to (16).

General procedure for the preparation of chlorhydrins 6

To a solution of 6.4×10^{-3} mole of the acetylenic compound in 25 ml THF maintained at -60°C under nitrogen, is added one molar equivalent (1.2 in the case of amine 4, 2.4 in the case of 2-methyl 3-butyne 2-ol) of a 1.6M hexane solution of butyllithium. The temperature is raised to -10°C in 30 mn and then decreased to -60°C, before the addition of a solution of 6.1×10^{-3} mole of the α-chloro carbonyl compound 5 in 5 ml of THF. The temperature is raised to 20°C in 120 mn. The solution is diluted with ether, and hydrolyzed by 10 ml of a saturated aqueous solution of NH_4Cl. The organic layer is washed with a minimum quantity of a saturated solution of NaCl, and then dried over $MgSO_4$. Removal of the solvent gives the chlorhydrins pure enough to be used in the next step. In certain case, purification of a sample is made by recrystallisation.

5-Amino-1-chloro 2,5-dimethyl 3-hexyne 2-ol 6a
Yield : 85%
F = 38-40°C (Ether)
IR : 3340, 3270, 3160, 2230, 1590, 1360, 1080, 740.
NMR (CCl_4 ; 60MHz) : 1.30 (s, 6H) ; 1.40 (s, 3H) ; 2.90 (s, exch., 3H) ; 3.40 (s, 2H).
6-Amino-2-chloro-3,6 dimethyl-4-heptyne 3-ol 6b
Yield : 93%
IR : 3340, 3280, 3170, 2240, 1590, 1380, 920, 700.
NMR (CCl_4, 60MHz) : 1.30 (s, 6H) ; 1.35 (s, 3H) ; 1.50 (d, J=6.5, 3Hz) ; 2.60 (s, exch. 3H) ; 3.85 (q, J=6.5Hz, 1H)
5-Amino 1-chloro ,5-methyl-2-phenyl 3-hexyne 2-ol 6c.
Yield : 80%
F = 116-118°C (chloroform)
IR ($CHCl_3$) : 3560, 3360, 3290, 3060, 2230, 1590, 1350, 1290, 1060, 880, 700.
RMN ($CDCl_3$, 60MHz) : 1.40 (s, 6H) ; 2.90 (s, exch., 3H) ; 3.60 (s, 2H) ; 7.15 to 7.80 (m, 5H).
5-Amino 1-chloro 5-methyl-3-hexyne 2-ol 6d
Yield : 94%
IR : 3340, 3280, 3170, 2240, 1600, 1380, 1230, 1110, 1070, 760.
NMR : ($CDCl_3$; 60MHz) : 1.40 (s, 6H) ; 3.30 (s, exch., 3H) ; 3.55 (d, J=6Hz, 2H) ; 4.50 (t, J=6Hz, 1H).
2-Chloro 3-methyl 4-nonyne 3-ol
Yield : 96%

IR : 3440, 2240, 1050, 920.

NMR (CCl_4, 60MHz) ; 0.90 (t, J=7Hz, 3H) ; 1.10 to 1.60 (m, 4H) ; 1.40 (s, 3H) ; 1.50 (d, J=6Hz, 3H) ; 1.95 to 2.50 (M, 3H) ; 3.85 (q, J=6Hz, 1H).

2-Chloro-3,6,6 trimethyl 4-heptyne 3-ol

Yield : 97%

IR : 3420, 2260, 2230, 1270, 1075, 1050, 920, 770, 700.

NMR (CCl_4, 60MHz) : 1.20 (s, 9H) ; 1.40 (s, 3H) ; 1.50 (d, J=7Hz, 3H) ; 2.65 (s, exch., 1H) ; 3.90 (q, J=7Hz, 1H).

2-Chloro 3,6-dimethyl-4-heptyne 3,6 diol

Yield : 73%

IR : 3420, 1175, 955, 860.

NMR ($CDCl_3$, 60MHz) : 1.35 (s, 3H) ; 1.40 (s, 6H) ; 1.60 (d, J=7Hz, 3H) ; 2.95 (s, exch., 2H) ; 4.05 (q, J=7Hz, 1H).

3-Chloro 5-decyne 4-ol

Yield : 95%

IR : 3380, 2240, 1060, 1030.

NMR (CCl_4, 60MHz) (mixture of both diastereoisomers) : 1.05 (t, J=7Hz, 6H) ; 1.20 to 2.00 (M, 6H) ; 2.00 to 2.35 (M, 2H) ; 2.60 à 3.05 (M, exch., 1H) ; 3.45 to 3.95 (m, 1H) ; 4.15 to 4.45 (m, 0.8H) ; 4.75 to 4.95 (0.2H).

General procedure for the preparation of epoxides.

To a vigorously stirred solution of 5.5×10^{-3} mole of the chlorhydrin in 30 ml of anhydrous ether are added at -20°C, 7.2×10^{-3} mole of potassium t-butylate. The temperature is raised to 20°C in one hour and the solution is washed by 3x10 ml of a saturated solution of NaCl, dried over MgSO4 and the ether is removed in vacuo. The epoxide is then isolated by bulb to bulb distillation at T° under pressure.

2,5-Dimethyl 1,2-epoxy-3-hexyne 5-amine 3a

Yield : 78%

T = 150°C P = 20 Torrs

G.C : t_R = 2.9 mn (col A, θ = 110°)

IR : 3360, 3290, 3040, 2240, 1595, 1380, 1335, 1270, 860, 800.

NMR ^1H ($CDCl_3$, 350MHz) : 1.36 (s, 6H) ; 1,52 (d, ^4J = 2Hz, 3H) ; 1.70 (s, exch. 2H) ; 2.73 (d, ^2J= 5.6Hz, 1H) ; 2.96 (dxq, ^2J=5.6Hz, ^4J=2Hz, 1H).

NMR ^{13}C ($CDCl_3$, 88MHz) : 23.20 (q) ; 31.57 (q) ; 44.92 (s) ; 46.92 (s) ; 55.06 (t) ; 79.00 (s) ; 89.81 (s).

M.S. m/z(%) : 139 (1, M+) ; 138 (2) ; 124 (100) ; 108 (15) ; 94 (31) ; 82 (14) ; 67 (17) ; 58 (9) ; 42 (34).

Because of the biological activity of 3a, this compound was converted to its solid N-acetyl derivative in order to have a correct centesimal analysis : to a solution of 0.326 g (2.35×10^{-3} mole) of 1a in 5 ml CH_2Cl_2 cooled to 0°C are added successively 0.65 ml of NEt_3 and 0.482 g (4.7x10-3 mole) of acetic anhydride with a crystal of DMAP. The temperature is raised to 20°C and the mixture is stirred 4 hours and hydrolyzed. Extraction with ether, usual work-up and recrystalisation in ether lead to 0.289 mg (68%) of the acetamide.

F = 91-93°C (Ether)

IR ($CHCl_3$) : 3440, 1680, 1500.

NMR (CCl_4, 60MHz) : 1.50 (s, 3H) ; 1.55 (s, 6H) ; 1.90 (s, 3H) ; 2.65 (d, ^2J=5Hz, 1H) ; 2.95 (d, ^2J=5Hz, 1H) ; 6.20 (s, 1H).

M.S (m/z, %) : 181 (3, M+) ; 166 (6) ; 151 (13) ; 136 (15) ; 124 (18) ; 110 (20) ; 95 (30) ; 94 (23) ; 54 (15) ; 52 (13) ; 45 (100) ; 44 (91) ; 43 (32) ; 41 (20) .

Anal. : ($C_{10}H_{15}NO_2$) : Calc. C 66.27 ; H 8.34 ; N 7.73 ; O 17.66.

found : C 66.21 ; H 8.30 ; N 7.54 ; O 17.95

2,5-Dimethyl 5,6-epoxy 3-heptyne -2-amine 3b

Yield : 45%

T = 150°C P = 20 Torrs

G.C : t_R trans = 2.9 mn (4%) ; t_R cis = 3.9 mn (96%) (col.A, θ =110°C)

IR : 3360, 3300, 2220, 1600, 1270, 1070, 850.

NMR ^1H ($CDCl_3$, 350 MHz) (R*S* isomer) : 1.30 (d, J=5.6Hz, 3H) ; 1.38 (s, 6H) ; 1.46 (s, 3H) ; 1.76 (s, exch., 2H) ; 3.21 (q, J=5.6Hz, 1H).

NMR ^{13}C ($CDCl_3$, 88MHz) (R*S* isomer) : 13.60 (q) ; 18.46 (q) ; 31.66 (q) ; 45.08 (s) ; 50.82 (s) ; 60.53 (d) ; 80.59 (s) ; 89.36 (s).

G.C - M.S (M/z, %) : (R*R* isomer) : 153 (, M+) ; 152 (4) ; 108 (15) ; 95 (10) ; 94 (100) ; 93 (11) ; 69 (17) ; 53 (10) ; 44 (15) ; 43 (28) ; 42 (73) ; 41 (31) ; 39 (21) ;. (R*S* isomer) : 153 (, M+) ; 152 (3) ; 138 (16) ; 108 (15) ; 94 (100) ; 93 (10) ; 91 (11) ; 53 (11) ; 51 (11) ; 44 (17) ; 43 (36) ; 41 (26) ; 40 (11) ; 39 (25).

High resolution (run on the mixture) : Calc. for $C_8H_{14}NO$ (M-H) : 152. 1074 ; found : 152. 1074.

1,2-Epoxy 5-methyl 2-phényl -3-hexyne 5-amine 3c

Yield : 45%

T = 140°C - P = 0.1 Torr

IR : 3360, 3290, 3060, 2240, 1600, 1300, 890, 760, 700.

NMR ($CDCl_3$, 350 MHz) : 1.44 (s, 6H) ; 1.82 (s, exch., 2H) ; 2.97 (d, 2J = 6.3Hz, 1H) ; 3.37 (d, 2J = 6.3 Hz, 1H) ; 7.28 to 7.49 (m, 5H) .

M.S (m/z, %) : 201 (6, M+) ; 200 (30) ; 186 (36) ; 171 (22) ; 170 (49) ; 156 (100) ; 129 (33) ; 128 (36) ; 115 (47) ; 105 (32) ; 77 (30) ; 58 (55) ; 51 (23) ; 42 (84).

1,2-Epoxy 5-methyl 3-hexyne 5-amine 3d

Yield : 5%

T = 100°C - P = 0.05 Torr

IR : 3360, 3290, 3050, 2240, 1600, 1370, 1220, 870, 820.

NMR ($CDCl_3$, 350 MHz) : 1.39 (s, 6H) ; 2.07 (s, exch., 2H) ; 2.85 (dxd, 2J = 5.6Hz, Jcis = 2.8Hz, 1H) ; 2.90 (dxd, 2J = 5.6Hz, J_{trans} = 4.2 Hz, 1H) ; 3.36 (dxd, J_{trans} = 4.2Hz, J_{cis} = 2.8Hz, 1H).

2,3-Epoxy 3-methyl 4-nonyne 7

Yield : 68%

T = 130°C - P = 20 Torrs

t_R trans = 3.4 mn (4%) ; t_R cis = 4.5 mn (96%) - (col. A, θ = 90°C)

IR : 3000, 2240, 1260, 1075.

NMR ^1H ($CDCl_3$, 350MHz) (R*S* isomer) : 0.90 (t, J = 7.1Hz, 3H) ; 1.29 (d, J=5.5Hz, 3H) ; 1.36 to 1.51 (m, 4H) ; 1.46 (s, 3H) : 2.18 (t, J=7.1Hz, 2H) ; 3.20 (q, J=5.5Hz, 1H).

NMR ^{13}C ($CDCl_3$, 50MHz) (R*S* isomer) : 13.37 (q) ; 13.44 (q) ; 18.1 (t) ; 18.4 (q) ; 21.7 (t) ; 30.4 (t) ; 50.9 (s) ; 60.3 (d) ; 80.87 (s) ; 82.3 (s).

G.C - M.S (m/z, %) (R*R* isomer) : 137 (9, M-Me) ; 110 (10) ; 109 (11) ; 93 (60) ; 91 (29) ; 79 (44) ; 77 (37) ; 66 (18) ; 53 (15) ; 51 (13) ; 43 (100) ; 41 (44) ; 40 (20) ; 39 (90). (R*S* isomer) : 137 (88, M-Me) ; 109 (10) ; 93 (54) ; 91 (28) ; 79 (42) ; 77 (38) ; 66 (22) ; 53 (15) ; 51 (13) ; 43 (100) ; 41 (42) ; 40 (20) ; 39 (87).

5,6-Epoxy 2,2,5-trimethyl 3-heptyne 8

Yield : 76%

T = 120°C - P = 20 Torrs

G.C : t_R trans = 3.0 mn (5%) ; t_R cis = 3.4 mn (95%) - (col.B, θ = 100°C)

IR : 3000, 2290, 2230, 1290, 1075, 890, 850, 770, 720.

NMR ^1H ($CDCl_3$, 300 MHz) (R*S* isomer) : 1.26 (s, 9H) ; 1.35 (d, J=5.5Hz, 3H) ; 1.50 (s, 3H) ; 3.22 (q, J=5.5Hz, 1H)

NMR ^{13}C ($CDCl_3$, 75 MHz) (R*S* isomer) : 13.65 (q) ; 18.68 (q) ; 27.20 (s) ; 30.87 (q) ; 50.84 (s) ; 60.40 (d) ; 79.46 (s) ; 90.33 (s).

G.C - M.S (m/z, %) trans isomer : 137 (10, M-Me) ; 108 (6) ; 93 (74) ; 91 (73) ; 79 (23) ; 77 (100) ; 53 (23) ; 51 (22) ; 43 (59) ; 41 (50) ; 39 (52). cis isomer : 137 (11, M-Me) ; 108 (12) ; 93 (95) ; 91 (82) ; 79 (26) ; 77 (100) ; 53 (22) ; 51 (20) ; 43 (74) ; 41 (59) ; 39 (55).

2,5-Dimethyl 5,6-epoxy 3-heptyne 2-ol 9

Yield : 70%

T = 100°C - P = 1 torr

t_R trans = 4.0 mn (15%) ; t_R cis = 4.6 mn (85%) - (col.B, θ = 120°C)

IR : 3420, 1270, 1170, 1075, 950, 850.

NMR ^1H ($CDCl_3$, 300 MHz) : 1.30 (d, J=5.5Hz, 2.55H) ; 1.41 (d, J=5.2Hz, 0.45H) ; 1.46 (s, 2.55H- ; 1.48 (s, 5.10H) ; 1.50 (s, 1.35H) ; 2.98 (q, J=5.2Hz, 0.15H) ; 3.23 (q, J=5.5Hz, 0.85H) ; 3.91 (s, exch., 1H).

NMR ^{13}C ($CDCl_3$, 75MHz) ; R*R* isomer : 15.44 (q) ; 23.26 (q) ; 31.36 (q) ; 52.81 (s) ; 60.88 (d) ; 64.68 (s) ; 79.16 (s) ; 89.51 (s). R*S* isomer : 13.50 (q) ; 18.21 (q) ; 31.28 (q) ; 50.89 (s) ; 60.45 (s) ; 64.59 (s) ; 82.12 (s) ; 86.83 (s).

G.C - M.S (m/z, %) : R*R* isomer : 110 (5, M-C_2H_4O) ; 95 (18) ; 67 (5) ; 51 (5) ; 43 (100) ; 41 (13) ; 39 (9). R*S* isomer : 110 (6, M-C_2H_4O) ; 95 (17) ; 67 (5) ; 43 (100) ; 41 (14) ; 39 (10).

3,4-Epoxy 5-decyne 10

Yield : 57%

T = 140°C - P = 20 Torrs

t_R cis = 5.1 mn (20%) ; t_R trans = 5.5 mn (80%) - (col.B, θ = 120°C)

IR : 2240, 880

NMR (CCl_4, 60MHz) : 0.65 à 1.20 (m, 6H) ; 1.20 to 1.85 (M, 6H) ; 1.95 to 2.40 (M, 2H) ; 2.70 to 2.95 (m, 11.8 H) ; 3.15 (dxt, J_{cis} = 4Hz ; ^6J = 1.5Hz, 0.2H).

G.C - M.S (m/z, %) : cis isomer : 152 (1, M+) ; 123 (3) ; 109 (1) ; 95 (12) ; 81 (23) ; 79 (100) ; 77 (37) ; 57 (21) ; 53 (21) ; 52 (45) ; 51 (30) ; 42 (26) ; 41 (55) ; 39 (71). trans isomer : 152 (1, M+) ; 123 (3) ; 109 (4) ; 95 (12) ; 81 (18) ; 79 (100) ; 77 (42) ; 66 (21) ; 65 (19) ; 57 (17) ; 55 (20) ; 53 (21) ; 52 (46) ; 51 (30) ; 42 (28) ; 41 (48) ; 39 (62).

Biological tests

Effect of synthetic products on cell growth

The cells used were : human embryonic lung fibroblasts MRC5 (kind gift of Institut Mérieux Lyon) ; normal rat kidney cells NRK, NRK transformed by the Prague strain of Rous Sarcoma Virus B77 and HeLa, a human epitheloïd carcinoma of the cervix (all obtained from the American Tissue Culture Collection) ; Hep2 - a cell line derived from a carcinoma of human larynx ; 293 : a cell line derived from human embryonic kidney transformed by the DNA of Adenovirus 5.

2.10^5 cells were seeded into 6 cm plastic Petri dishes containing 5 ml of Eagle's minimum essential medium containing glutamine supplemented with 10% new born calf serum. Cells were incubated at 37°C in a humid atmosphere of air + CO_2 (95/5). 4h after seeding, the compounds to be studied, in solution in 0.14 M aqueous NaCl were added at the concentrations indicated in table 3. Controls contained 0.14 M aqueous NaCl alone or equivalent amount of solvent if the product was not water soluble. After 4 days in culture, cells were harvested and the effect on growth determined either by direct cell count or by protein content. Results are expressed as percentage inhibition compared to solvent treated controls.

Determination of malondialdehyde

Malondialdehyde was measured in the form of its thiobarbituric acid derivative by its O.D. at 532 nm according to the method described previously (3). For microquantities of malondialdehyde 1 nmol) the thiobarbituric derivative was measured by its fluorescence λ ex 532 nm ; λ em 553nm as described (17).

Enzymatic assays

Preparation of homogenates from cells in tissue culture : the cell sheets were rinsed twice with cold 0.14 M aqueous NaCl after which the cells were scraped off with a rubber-covered rod, transferred to tubes and washed by centrifugation at 600 g for 5 mn. The cell pellet was stored at -20°C until use. Cells were disrupted by sonication in a Bronson sonifier for 2 sec at output 2 with a microprobe and finally suspended in 0.14 M aqueous NaCl.

Assay of aldehyde reductase : To 1 ml of cell homogenate from NRKB77 cells containing 5.0 mg protein was added compound 3a in three separate doses of 200, 800 and 1000 nmol/ml. After incubation for 15, 30 and 60 mn, an aliquot of 100 µl containing 500 µg protein was added to a micro cuvette containing 0.3 ml of 0.1M phosphate buffer pH6, the substrate malondialdehyde at 2 mmole and the cofactor NADPH at 35 mmole and brought to a final volume of 1 ml with water.

The contents of the cuvette were rapidly mixed by inversion, and the O.D. noted. This gave the "0" time O.D. Readings were made at 1 mn intervals for a total of 5 mn. Inhibition was assessed by the decrease in O.D./mn between homogenates preincubated with inhibitor (experimental) and homogenates without inhibitor (control).

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