[M.p. 198-199 °C; $[\alpha]_D$ ($CHCl_3$) = 11.6°; UV (MeOH) 234 (6800) nm; IR ($CHCl_3$) 3500, 1725, 1650 cm^{-1}; MS 416 (M^+); PMR (C_6D_6) 6.24 (H-16, m; w/2 10Hz), 3.92 (H-12, dd, J = 4, 10Hz), 3.36 ($-OCH_3$, s), 3.06 [CH=C−CH_2−CO, ABq, J = 15Hz, 2H long-range coupled with H at C(16)]; the CMR data are reported together with those of the acetyl derivative **11** in the table].

The above arguments lead to the allocation of the structure **5** to the new sesterterpenoid. The localization of the acetoxy group on C(16) is suggested by the easy elimination, observed in the mass spectrum of acetic acid, which could not occur in the alternative structure **(12)** showing the acetoxy group on C(18). The relative stereochemistry of scalarolbutenolide has been established, as shown in formula **5**, according to previous findings for scalarin-like compounds[4,5,8,9] along with the configurational arguments reported throughout the text. The Horeau method[10] applied to **5** allows to determine the chirality of C(12) as R, and this determines its absolute stereochemistry.

Scalarolbutenolide shows the same tetracarbocyclic skeleton of the scalarin-like compounds with different arrangements of the carbons C(19) and C(20). The same skeleton as **5** has been previously encountered only in furoscalarol **(13)**[11,9].

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2 We are indebted to A. Crispino, C. Di Pinto, G. Scognamiglio and R. Turco for their technical assistance.
3 E. Fattorusso, L. Minale, G. Sodano and E. Trivellone, Tetrahedron *27*, 3909 (1971).
4 G. Cimino, S. De Stefano, L. Minale, and E. Trivellone, J. chem. Soc. Perkin I, *1977*, 1587.
5 G. Cimino, S. De Stefano and A. Di Luccia, Experientia *35*, 1277 (1977).
6 E. Fattorusso, S. Magno, C. Santacroce and D. Sica, Tetrahedron *28*, 5993 (1972).
7 E.R.H. Jones, 8th Symp. Chem. Natural Products, New Delhi, February 1972, p. 43. Ed. T.R. Govindachari. Butterworths, London 1972.
8 Y. Kashman and A. Rudi, Tetrahedron *33*, 2997 (1977).
9 G. Cimino, F. Cafieri, L. De Napoli and E. Fattorusso, Tetrahedron Lett. *1978*, 2041.
10 A. Horeau, Tetrahedron Lett. *1961*, 506; *1962*, 965.
11 F. Cafieri, L. De Napoli, E. Fattorusso, C. Santacroce and D. Sica, Gazz. chim. ital. *1977*, 1587.

The synthesis of enantiomers of 4-ketocyclophosphamide

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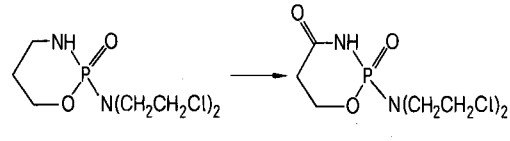
Summary. Oxidation with Fenton's reagent and by direct ozonation of the enantiomers of the antitumour agent cyclophosphamide afforded optically pure enantiomers of 4-ketocyclophosphamide. Oxidation with potassium permanganate, however, gave the corresponding racemate.

As part of an investigation into the stereoselective metabolism of cyclophosphamide (**1**) and its congeners in experimental animals[1] and in man[2], a study of the stereospecific synthesis of the parent drugs[3] and their metabolites was undertaken. In this communication we report on the synthesis of the enantiomers of 4-ketocyclophosphamide (**2**). Cyclophosphamide (**1**) is readily oxidized by $KMnO_4$ to **2**[4], but when cyclophosphamide enantiomers were similarly treated, only racemic **2** was obtained.

However, oxidation of optically pure $R(+)$-**1** with Fenton's reagent ($FeSO_4/H_2O_2$)[5] afforded $R(-)$-**2** as the thick oil in 4.5% yield $|\alpha|_D^{25} = -30°$; unreacted $R(+)$-**1** with unchanged optical rotation was recovered. Although the chemical and optical purity of the above product **2** has been proved by ^{31}P NMR spectroscopy in the presence of Eu(tfc)$_3$ reagent, by mass spectrometry and by several chromatographic techniques, attempts to crystallize it have failed. For purposes of comparison 4-ketocyclophosphamide was prepared by ozonation[6] of $S(-)$-**1**. The crystals of $S(+)$-**2** m.p. 107 °C (from ethyl-ether), were obtained $|\alpha|_D^{25} = +53.8°$ (c 3, MeOH) in 16% yield. The enantiomeric purity of at least 97% was demonstrated using the ^{31}P NMR/Eu(tfc)$_3$ technique. Because the absolute configuration of the starting cyclophosphamide was known[7], and no bond to the chiral phosphorus atom was cleaved, the absolute configuration of (+)-**2** ($|\alpha|_D^{25} = +53.8°$) is *S*. It should also be emphasized that oxidation by Fenton's reagent is a process which mimics in some respects the biological oxidations mediated by the liver mixed function oxidases[8]. It would therefore be predicated that racemization of enantiomeric cyclophosphamides should not occur during oxidative metabolism.

$S(+)$-4-Ketocyclophosphamide $|S(+)$-**2**$|$. Gaseous ozone (Ozon Generator, Fischer 501) was bubbled at 0 °C at a rate of 1 g/h through a solution of $S(-)$-cyclophosphamide (**1**, $|\alpha|_D^{25} = -2.3°$, 3.83 mmole) in acetone (9 ml) and water (18 ml). After 8 h the ^{31}P NMR spectrum of reaction mixture revealed the presence of **2** (20%, $\delta = 6.5$ ppm), 4-hydroperoxycyclophosphamide (20%, $\delta = 10.2$ ppm) and unreacted **1** (60%, $\delta = 14.3$ ppm). Further ozone did not change the composition of the mixture. After evaporation of the acetone, the aqueous solution was extracted with chloroform (3 × 25 ml). The product was isolated by column chromatography on silica gel with chloroform-acetone (1:1) as an eluent. $S(+)$-**2** was recrystallized from ethyl ether to obtain 168 mg (16% yield) as white crystals |m.p. 107 °C $|\alpha|_D^{25} = +53.8°$ (c 3, MeOH), $R_f = 0.59$ (chloroform-acetone 1:3), $\delta_{31P} = 5.0$ ppm ($CHCl_3$, downfield from



1 2

H_3PO_4), MS: m/z 275 ($M^{+\cdot}+1$, 1%), 225 (100%), 92 (52%), 55 (58%)|. The unreacted *S*(−)-**1** |checked by ^{31}P NMR and MS, $R_f=0.41$ (chloroform-acetone, 1:3)| $|\alpha|_D^{25}=+2.3°$.

^{31}P NMR spectrum of racemic **2** in the presence of $Eu(tfc)_3$ molar ratio **2**:$Eu(tfc)_3$ = 1:0.5 in $CDCl_3$ solution (2 ml, conc. 0.044 mole/l) revealed the presence of 2 signals at $\delta_-=-25.4$ ppm and $\delta_+=-29.4$ ppm in 1:1 ratio. The ^{31}P NMR spectrum of enantiomer S(+)-**2** (conc. 0.022 mole/l, other conditions as above) indicated the presence of single peak at $\delta=-26.9$ ppm. Addition of racemic **2** caused an increase of intensity of the peak at $\delta=-26.9$ ppm and the appearance of a 2nd signal at $\delta=-23.7$ ppm. The accuracy of measurement was established as ±3% on the basis of results obtained with samples of known (by weight) enantiomeric composition.

R(−)-4-Ketocyclophosphamide |*R*(−)-**2**|. To a solution of *R*(+)-cyclophosphamide (**1**, $|\alpha|_D^{25}=+2.3°$. 261 mg, 1 mmole) and $FeSO_4\cdot 7\ H_2O$ (556 mg, 2 mmole) in water (10 ml) were added, with stirring, 5% H_2O_2 (1.36 ml, 2 mmole) and phosphate buffer (0.2 mole/l, 10 ml, pH = 7.5). After 1 h at room temperature the mixture (pH = 3–4) was extracted with $CHCl_3$ (3 × 25 ml). Evaporation of solvent left 131 mg of oily liquid. Preparative TLC in chloroform-acetone (1:3) was used to separate unreacted substrate and *R*(−)-**2** |12.4 mg (4.5 yield), $|\alpha|_D^{25}=-30\pm1°$ (c 1.2, MeOH), other data are identical as for *S*(+)-**2**|.

1 P.J. Cox, P.B. Farmer, A.B. Foster, L.J. Griggs, M. Jarman, R. Kinas, K. Pankiewicz and W.J. Stec, Biomed. Mass Spectrom. *4*, 371 (1977).
2 M. Jarman, R.A.V. Milsted, J.F. Smyth, R.W. Kinas, K. Pankiewicz and W.J. Stec, Cancer Res. *39*, 2762 (1979).
3 W.J. Stec, R.W. Kinas and K. Pankiewicz, Bull. Acad. pol. Sci., Sér. Sci. chim. *23*, 981 (1975).
4 M. Jarman, Experientia *29*, 812 (1973).
5 J. van der Steen, E.C. Timmer, J.G. Westra and C. Benckhuysen, J. Am. chem. Soc. *95*, 7535 (1973); R.F. Struck, M.C. Thorpe, W.C. Coburn, Jr, W. Russel Laster, Jr, J. Am. chem. Soc. *96*, 313 (1974).
6 H.J. Hohorst, G. Peter and R.F. Struck, Cancer Res. *36*, 2278 (1976).
7 D.A. Adamiak, W. Saenger, R.W. Kinas and W.J. Stec, Angew. Chem. *89*, 336 (1977).
8 S. Udenfriend, C.T. Clark, J. Axelrod and B.B. Brodie, J. biol. Chem. *208*, 731 (1954); R.M. Rauen, K. Norpoth and E. Golovinsky, Naturwissenschaften *54*, 589 (1967).

Incorporation of α-aminobutyric acid into ergostine by *Claviceps purpurea*

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Summary. Claviceps purpurea 275 F.I. fed with α-aminobutyric acid produces ergostine with the corresponding α-hydroxy-α-aminoacid undiluted by endogenous aminoacid. Almost all the α-aminobutyric acid not incorporated into the ergostine remains unchanged in the medium. The significance of these results in the biosynthesis of ergot alkaloids is discussed.

In spite of the many studies performed on the biosynthesis of ergot peptide alkaloids, it is not yet clear how the peptide side chain adds to the lysergyl moiety. These studies are hampered by the ability of the employed strains of *Claviceps* to cleave the lysergylvaline or lysergylalanine in lysergic acid and the corresponding aminoacid moiety[1–3]. The labelling of these precursors therefore does not help in the determination of the biosynthetic pathway, because the 2 aminoacids released are incorporated through many metabolic pathways[1,4]. In the side chain of ergostine (**1**) the aminoacid present in the first position is α-aminobutyric acid (ABA), in the second one phenylalanine and in the third proline.

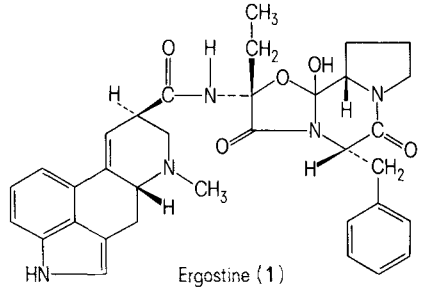
C. purpurea 275 F.I. produces mainly ergotamine and small amounts of ergokryptine but does not produce ergostine.

In a previous work[5] we have shown the non specificity of the enzyme catalyzing the synthesis of the peptide side chain of ergot alkaloids. In fact several aminoacids, or their analogues, structurally related to the second aminoacid of the peptide side chain, are incorporated into the alkaloids when added to the cultures. If the addition of ABA in the medium enables *C. purpurea* 275 F.I. to produce ergostine, it is reasonable to conclude that the aminoacid is directly incorporated into the alkaloid by substituting alanine and thus shifting the production from ergotamine to ergostine. Furthermore, ABA is seldom found in nature, scarcely metabolized and therefore very suitable for labelling studies since its distribution and incorporation is limited to the target compound.

In the present work we report the results obtained by examining the incorporation of ABA in the first position of the peptide side chain and in particular the production of ergostine in fermentation broths of *C. purpurea* 275 F.I. when ABA is added to the culture medium. By using labelled ABA it has been also possible to follow the fate of this aminoacid in the culture.

Materials and methods. Claviceps purpurea 275 F.I., a producer of ergotamine and ergokryptine, was grown using the media and conditions published elsewhere[6]. Two 35 ml cultures were supplemented each with 17.5 mg of ABA-(3-^{14}C), sp. act. 125.8 nCi/μmole, on the 7th day, and were harvested 7 days later.

The cultures, after addition of an equal volume of acetone and 1% of solid tartaric acid, were homogenized and filtered, and the extract evaporated to dryness. The residue,



Ergostine (1)