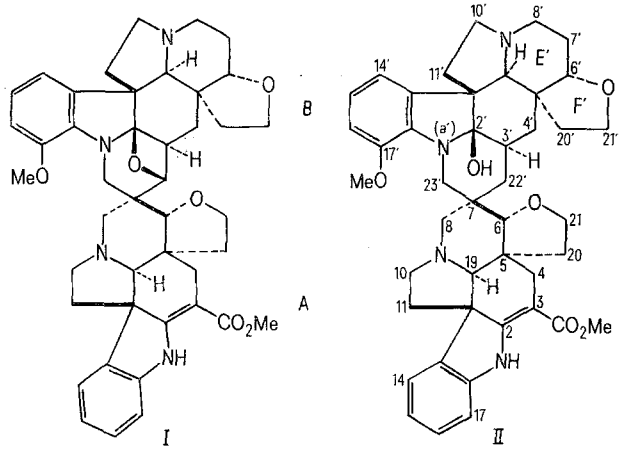
atoms. The MS-spectrum accounts for an unaltered A portion of the molecule (ions at *m/e* 305 and 363)[5], while the fragments at *m/e* 393 and 504 of the vobtusine spectrum shift at *m/e* 391 and 502 respectively, showing that the additional ring is present in part B. Furthermore, the MS (ions at *m/e* 110 and 138)[5] indicates that the F′ and E′ rings do not carry substituents.



From these data it is clear that the carbon involved in the ethereal bridge can only be one of the atoms C-22′, C-3′, C-4′ and C-11′, the absence of low-field resonating singlets in the ^{1}H-NMR-spectrum of I automatically excluding C-23′ as a possible linkage position.

Comparison of the ^{13}C-NMR-spectrum of quimbeline (22.6 MHz, $CDCl_3$) with that of vobtusine gives support for the location of the ethereal bridge at C-22′. In addition to the signal of the β-anilino-acrylic ester unit (C-2 166.9, C-3 93.9, C-13 137.7[a], C-14 121.5, C-15 120.6, C-16 127.7, C-17 109.3, C-18 143.1, C=O 168.6, OCH_3 51.0) and the 7-methoxyindoline grouping (C-13′ 137.4[a], C-14′ 114.8, C-15′ 118.3, C-16′ 111.0, C-17′ 145.2, C-18′ 134.5, OCH_3 55.1), the spectrum of vobtusine exhibits a strongly deshielded singlet at δ 94.4 due to the C-2′ atom, which confirms that the hydroxy function is part of a carbinolamine system. The chemical shift assignments for the vobtusine carbon atoms derive from shift theory, and partially follow the arguments discussed by WENKERT[6] in the ^{13}C-NMR analysis of *Aspidosperma* alkaloids:

C-6	87.6	C-6′	80.5
C-19	69.0	C-19′	63.7
C-21	64.3[b]	C-21′	65.3[b]
C-12	54.9	C-12′	56.0
C-8	53.9	C-8′	48.8
C-10	51.9[c]	C-10′	51.0[c]
		C-23′	46.2[d]
C-5	47.7	C-5′	44.2
C-11	45.0[d]	C-11′	36.5
C-7	39.7	C-7′	27.4
C-20	34.8	C-20′	33.9
C-4	25.8		

[a, b, c, d] Assignments may be reversed.

The remaining three carbon atoms (C-22′, C-3′ and C-4′) resonate at δ 31.2, 31.5 and 32.5.

The ^{13}C-NMR-spectrum of quimbeline is the same in character as that of vobtusine, the most noteworthy differences being the presence of an additional oxymethyne carbon atom at δ 82.0 and the downfield shift of the C-7 singlet at δ 43.3 and of the C-3′ doublet at δ 58.3. These data are only compatible with the allocation of the ethereal bridge at C-22, as represented in formula I.

Since upon hydrogenolytic fissure of the oxetane ring[7] (MeOH, Pd-C, 5 days) vobtusine is obtained, the 2 alkaloids are assigned the same absolute stereochemistry.

Quimbeline is therefore the first compound belonging to the vobtusine class which surely possesses an oxetane ring. In fact, amataine should contain an analogous ring between C-2′ and C-23′, though an alternative ethereal bridge between C-2′ and C-8 cannot be excluded[3].

Résumé. La structure I a été attribuée à la quimbéline, un nouvel alcaloïde indolique extrait de l'écorce des racines du *Voacanga chalotiana*, grâce à des méthodes physiques et notamment à la comparaison de son spectre de résonance magnétique du ^{13}C avec le spectre de la vobtusine (II).

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20 June 1974.

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[8] Laboratorio Ricerche, Inverni della Beffa, Milano, Italy.
[9] Istituto di Chimica Organica dell'Università, Milano, Italy.

Properties of the Purified Penicillin V-Acylase of *Erwinia aroideae*

The activity of a penicillin V-acylase, (E.C. 3.5.1.11) present in *Erwinia aroideae* was studied. The bacterium was grown in shaking cultures on the following medium: glucose 1.0 g; secondary potassium phosphate 1.0 g; yeast extract 5 g; tryptone 5.0 g; distilled water 1 l (pH 7.0). After 24 h of growth at 28 °C, the cells were centrifuged, washed 3 times and suspended in a sterile phosphate buffer (pH 5.6). To the cell suspensions penicillin V (4 mg/ml final concentration) or other penicillins were added. The degradation products were determined qualitatively by thin layer chromatography[1] and quanti-

[1] E. J. VANDAMME and J. P. VOETS, J. Chromat. *71*, 141 (1972).

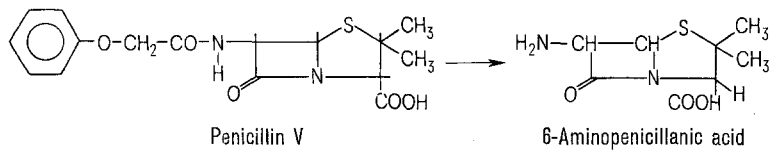

Fig. 1. Bioconversion of penicillin V into 6-aminopenicillanic acid by penicillin-acylase action.

tatively by the colorimetric method of SARGENT[2] or by the microbiological method of OOSTENDORP[3].

At pH 5.6 penicillin V is rapidly and specifically transformed into 6-aminopenicillanic acid (6-APA) by suspensions of intact cells[4] (Figure 1). No other degradation products could be detected. β-Lactamase is completely absent. The enzymatic activity is strictly associated with the cells and the penicillin V-acylase is not produced exogenously. At pH 5.6 only penicillin V is hydrolyzed into 6-APA; other penicillins or penicillin derivatives are not affected.

The hydrolytic activity of this *Erwinia* acylase on penicillin G and V is very low at pH 8.0, but cloxacillin and methicillin are transformed at an appreciable rate. Other semi-synthetic penicillins are not hydrolyzed at all at any pH value.

At pH 8.0 the amides isonicotinamide, benzamide, acetamide, L-glutamine, L-asparagine and urea are hydrolyzed by a dense suspension of *E. aroideae* cells. L-glutamine and L-asparagine are hydrolyzed at pH 5.6, while the other amides are not affected. Low transacylase activity is detected only with acetamide as substrate[4].

The transformation rate of penicillin V is influenced by the cell density in the reaction mixture; 5×10^{11} cells/ml being the optimum.

In the reaction mixture containing intact cells and penicillin V, the 6-APA produced, disappears partly upon further incubation. It was concluded that the 6-APA was transformed to some extent again into penicillin Vby resynthesis. Acetone dried cells are fully active in transforming penicillin V into 6-APA, but resynthetic activity seems to be destroyed. This would indicate that a different enzymatic activity is responsible for this resynthesis phenomenon.

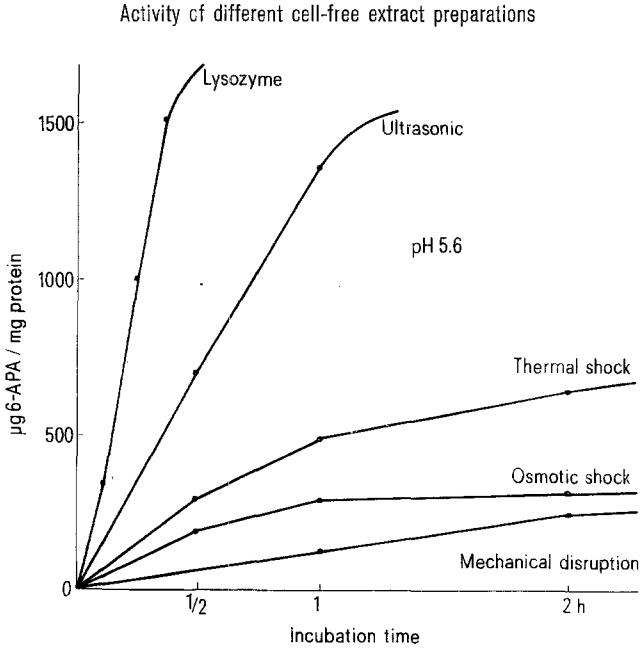

Fig. 2. Acylase-activity of different cell-free extract preparations (based on identical protein content) of *Erwinia aroideae*.

Cell-free extracts of *E. aroideae* were prepared by the following techniques: mechanically by grinding with ballotini glass in a Mickle apparatus or with a MSE ultrasonic disintegrator; by the osmotic shock procedure; by thermal shock procedure (10 min at 70 °C); enzymatically by treatment with lysozyme in the presence of EDTA. The enzymatic activities of these 5 extracts on penicillin V are shown in Figure 2. Treatment with lysozyme and ultrasonic disintegration seem to be the most effective procedures for the preparation of the enzymatic cell extracts. The activity of the cell-free extracts, prepared by thermal shock, indicates the heat stability of the acylase present. The results obtained suggest that the penicillin V-acylase is probably located near the cytoplasmic membrane.

For the preparation of purified cell-free extracts the following procedure was used. Washed cells were suspended in a mixture containing tris buffer (pH 8.0), EDTA 10^{-4} *M* and β-mercaptoethanol 0.5 m*M*. After homogenization, lysozyme was added to prepare a crude cell-free extract and the mixture was kept at 37 °C during 15 min. The viscous liquid was treated with 2% dihydrostreptomycine sulfate to remove nucleic acids. After clarification, the pale amber fluid was treated with ammonium sulfate. The resulting precipitate was dissolved in phosphate buffer M/15 at pH 5.6, containing 0.5 m*M* β-mercaptoethanol and dialyzed against the same buffer. The dialyzed fluid was eluted on a cellulose No. 123 column. The eluate was applied to a Sephadex G-25 column and eluted with the buffer. Fractions of 5 ml were collected. The active fractions were pooled and lyophilized. The concentrated Sephadex G25 effluent was applied to a Sephadex G100 column and eluted with phosphate buffer of pH 5.6, containing 0.5 m*M* β-mercaptoethanol. The fractions showing acylase activity were pooled and lyophilized. Figure 3 shows the distribution of the protein content and the acylase activity of the different fractions after elution on a Sephadex G100 column. The resulting white powder was stored at 4 °C. This preparation was used as the purified enzyme throughout the further experiments as this extract showed on cellulose acetate electrophoresis two bands both displaying acylase activity. On this criterion, this final preparation was estimated to be homogenous. The data obtained in a typical enzyme purification experiment are presented in Table I. The molecular weight of the purified enzyme as estimated by thin layer gel chromatography was ± 62.000.

The substrate specificity of the enzyme preparation was tested at pH 5.6 on the following compounds: penicillin V and G, their penicilloic and penilloic acids, methicillin, ampicillin, cloxacillin and oxacillin. Only penicillin V was

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[4] E. J. VANDAMME, J. P. VOETS and A. DHAESE, Ann. Inst. Pasteur *121*, 435 (1971).

Table I. Purification procedure on the *E. aroideae* acylase

Treatment	Volume	Total activity (unit)	Total protein (mg)	Specific activity (U/mg)	Purification	Recovery percentage
Lysozyme	150	1050	2250	0.4	1	100
Ammonium sulfate precipitation	80	930	1450	0.65	–	–
Cellulose No 123	50	750	810	0.93	–	–
Sephadex G25	20	380	340	1.2	–	–
Sephadex G25	15	150	44	3.5	–	–
Sephadex G100	5	146	3.7	39	98 times	14

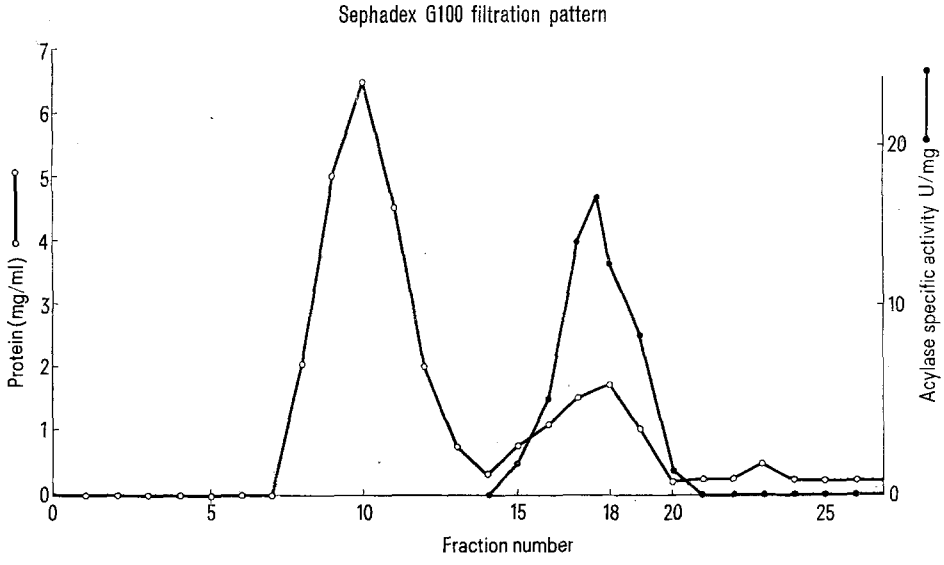

Fig. 3. Sephadex G100 filtration pattern of *Erwinia aroideae* penicillin V-acylase. ○—○, protein content; ●—●, acylase activity; 1 unit (U) is defined as the amount of enzyme, which produces 1 μmole of 6-APA under the conditions of the experiment.

transformed into 6-APA. Trace amounts of 6-APA were found from penicillin G. The activity of the purified enzyme extract was pH dependent, in the same way as the intact cell activity. The ability of the enzyme to catalyse the reverse action (penicillin resynthesis) was tested by adding sodium phenoxyacetate and 6-APA to the enzyme solution in the pH range 4 to 8. No measurable amounts of penicillin V could be detected.

With this purified enzyme however, tripeptides containing the glycine-glycine moiety were hydrolyzed at pH 5.6. Dipeptides, N-acetyl-L-amino acids or analogous compounds were not affected. The purified enzyme preparation was also examined for transacylase activity. The acylation of hydroxylamine by acids or amides to yield hydroxamic acids was not detected. This indicates that the structure of both the acyl and acylated part of the substrate influences strongly the rate of the hydrolytic action.

These specific patterns indicate that this penicillin V-acylase is not a non-specific acetylase or amidase as is suggested for many other penicillin acylases[5–10]. Not only the side chain structure of the penicillin V molecule, but also the intact β-lactam ring are necessary for full activity, as is proven by the lack of activity on phenoxymethylpenicilloic and phenoxymethylpenilloic acid. However, only recently penicillin acylases have been purified

Table II. Influence of tryptophane on acylase biosynthesis

Tryptophane concentration (%)	6-APA production (%)
0	100
0.001	96
0.01	100
0.05	96
0.1	98

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Table III. Influence of glucose and nitrogen sources on acylase biosynthesis

Glucose concentration (%)	6-APA production (%)	Nitrogen source (5 g/l)	6-APA production (%)
0	100	Tryptone	100
0.05	95	Peptone	100
0.10	100	Neopeptone	120
0.15	110	Casamino acids	100
0.25	105	Proteose peptone	100
		Vitamin-free casamino acids	100
		Casitone	100
		$(NH_4)_2SO_4$	100

and characterized[11–20] and a general picture of their substrate specificity is still difficult to indicate[21].

In order to find some indications about the metabolic function of this acylase, *E. aroideae* was grown in different media. According to GOTOVTSEVA[22] acylase biosynthesis by bacteria depends on the tryptophane content of the growth medium. The following basal medium was used for the growth of the organism: casamino acids 5 g; beef extract 7 g; glucose 1 g; secondary potassium phosphate 1 g; water 1 l. (pH 7.0) DL-tryptophane was added at concentrations of 0.001, 0.01, 0.05 and 0.1%. After 48 h at 28 °C ultrasonic extracts of the grown cells were prepared and checked for acylase activity. The results obtained are summarized in Table II. It is clear that the introduction of tryptophane in the growth medium has no influence on the acylase activity in this case.

The influence of glucose and different nitrogen sources on the production of the acylase was determined in the same way. The results are summarized in Table III. The presence or absence of glucose has no appreciable influence on the acylase biosynthesis. All nitrogen sources tested, behave in the same way as tryptone. Only neopeptone shows a slight stimulatory effect. From these results it can be concluded that the penicillin V-acylase, present in *Erwinia aroideae* cells is a constitutive enzyme.

This penicillin acylase presents properties aberrant from the classical penicillin acylases[23]. It is surely not a real 'bacterial' acylase and cannot be classified as a typical 'fungal' acylase. Indeed, its substrate spectrum and its pH optimum are quite unusual.

However, during this work was in progress a raising number of microbial penicillin acylases have been described differing in many aspects from the classical types[5,10,19,21,24].

Résumé. Une pénicilline V-acylase intracellulaire, produite par une souche *Erwinia aroideae* a été purifiée et caractérisée. Cette enzyme présente des propriétés différentes des penicilline-acylases classiques.

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Inhibitors of Prostaglandin Catabolism. I. Differential Sensitivity of 9-PGDH, 13-PGR and 15-PGDH to Low Concentrations of Indomethacin

Since the first reports that prostaglandin biosynthesis could be inhibited by aspirin-like drugs[1–5], considerable attention has been directed towards the use of these compounds in exploring the possible role of prostaglandins in the maintenance of body homeostasis. For example, through the use of these drugs prostaglandins have been implicated in the maintenance of smooth muscle tone[6,7], as mediators in neurotransmission[8–10], in autoregulation of renal blood flow[11–13] and in uterine contractility[14–16].

Indomethacin has been considered as a potent selective blocker of prostaglandin biosynthesis both in in vivo and in vitro systems. In this report, the first of a series in which we describe the inhibitory effect of certain drugs on the catabolism of prostaglandins, we demonstrate that