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.

E. J. VANDAMME and J. P. VOETS

Laboratory of General and Industrial Microbiology, University of Gent, Coupure 533, B–9000 Gent (Belgium), 29 August 1974.

[11] E. WALDSCHMIDT-LEITZ and G. BRETZEL, Hoppe Seyler's Z. physiol. Chem. *337*, 222 (1964).
[12] C. CHIANG and R. E. BENNETT, J. Bact. *93*, 302 (1967).
[13] N. S. BONDAREVA, M. M. LEVITOV and E. V. GORYACHENKOVA, Biokhimiya *34*, 96 (1969).
[14] D. A. SELF, G. KAY, M. D. LILLY and P. DUNNILL, Biotech. Bioeng. *11*, 337 (1969).
[15] B. SPENCER and C. MAUNG, Biochem. J. *118*, 29 P (1970).
[16] F. BAUMANN, R. BRUNNER and M. ROHR, Hoppe Seyler's Z. physiol. Chem. *352*, 853 (1971).
[17] D. WARBURTON, K. BALASINGHAN, P. DUNNILL and M. D. LILLY, Biochim. biophys. Acta *284*, 278 (1972).
[18] S. B. THADHANI, P. S. BORKAR and S. RAMACHANDRAN, Biochem. J. *128*, 49 P (1972).
[19] R. OKACHI and T. NARA, Agric. biol. Chem. *37*, 2797 (1973).
[20] C. KUTZBACH and E. RAUENBUSCH, Hoppe Seyler's Z. physiol. Chem. *355*, 45 (1974).
[21] E. J. VANDAMME and J. P. VOETS, Adv. appl. Microbiol. *17*, 311 (1974).
[22] V. A. GOTOVTSEVA, G. L. GORCHENKO and N. N. SUVOROV, Microbiologiya *37*, 683 (1968).
[23] J. M. T. HAMILTON-MILLER, Bact. Rev. *30*, 761 (1966).
[24] T. NARA, M. MISAWA, R. OKACHI and M. YAMAMOTO, Agric. biol. Chem. *35*, 1676 (1971).

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

indomethacin at concentrations known to inhibit prostaglandin biosynthesis, also inhibits to different degrees, each of three PG catabolizing enzymes recently shown to occur in the adult rat kidney[17].

Materials and methods. Kidneys (approx. 0.90 g) from male Wistar rats (200–250 g) were removed, freed from surrounding capsule, opened and washed thoroughly with ice-cold 0.05 *M* KH_2PO_4-NaOH (pH 7.4) buffer. The tissue was homogenized in 20 volumes buffer (Polytron, top speed, 10 sec, 0°). The incubation system contained homogenate (0.5 ml, approx. 8 mg protein), 9β-$^3H_1$-$PGF_{2\alpha}$ (2.2×10^6 dpm, S.A. 9.26 Ci/mole) to measure 15-PGDH and 13-PGR activities or $^3H_6$-15KD-$PGF_{2\alpha}$ (0.25×10^6 dpm S.A. 125 Ci/mole) to measure 9-PGDH activity[17], NAD^+ (final concentration 4 m*M*) and indomethacin (25 µl of a solution ranging from 0–1 mg/ml in buffer previously heated to 80 °C to dissolve the indomethacin). Incubations with $PGF_{2\alpha}$ lasted 10 min at 30 °C while with 15KD-$PGF_{2\alpha}$ 2.5 min at 30 °C. Incubations were terminated with 5 volumes of absolute ethanol. After centrifugation, the resulting supernatant was transferred and evaporated to complete dryness in vacuo. The residue was resuspended in 5 ml ethanol and aliquots were assayed both for total radioactivity in a liquid scintillation spectrophotometer (Beckman LS-255) and for radioactive products by thin layer radiochromatography as previously described[18].

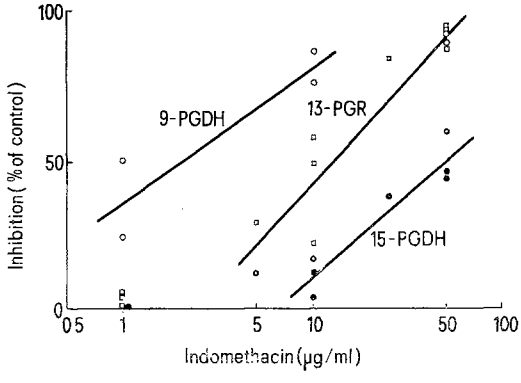


Fig. 1. Response of 9-PGDH, 13-PGR and 15-PGDH present in in adult rat kidney homogenates to increasing concentrations of indomethacin. Incubation system contained tracer prostaglandin, homogenate (0.5 ml, approx. 8 mg protein), NAD^+ (4 m*M* final concentration) and indomethacin. Each point represents a separate experiment. ○—○, 9-PGDH; □—□, 13-PGR; ●—●, 15-PGDH.

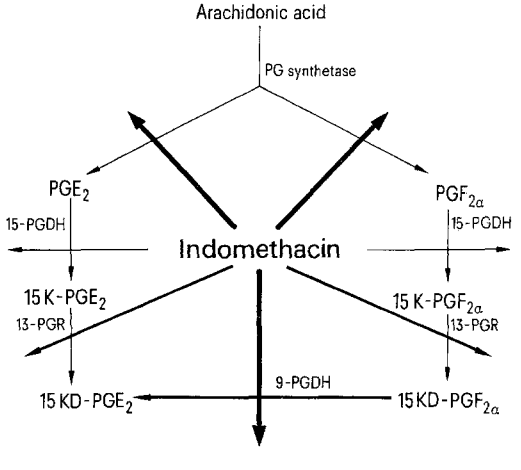


Fig. 2. Scheme of prostaglandin biosynthesis and catabolism showing sites of inhibition by indomethacin. Effectiveness of inhibition is represented by bars of varying width. 15K = 15-keto; 15KD = 15-keto-13,14-dihydro.

Results. The inhibitory effect of increasing concentrations of indomethacin on 9-PGDH, 13-PGR and 15-PGDH is shown in Figure 1. The order of sensitivity of these enzymes to indomethacin is 9-PGDH > 13-PGR > 15-PGDH. Concentrations of indomethacin required to produce 50% inhibition were approximately 2 µg/ml for 9-PGDH; 10 µg/ml for 13-PGR; 50 µg/ml for 15-PGDH. Thus at low concentrations of indomethacin, selective blockade of the transformation of the F metabolites into the E metabolites is achieved (see Figure 2). This should result in the accumulation of 15-keto-13,14-dihydro metabolites (E and F). At medium concentrations 15-keto metabolites should accumulate as a consequence of 13-PGR blockade while at high concentrations of indomethacin the primary prostaglandins should have a longer life time thereby eliciting a long lasting biological effect since both biosynthesis and catabolism are blocked. Although the latter effect is likely not applicable for consideration during therapeutic administration of the drug since such high concentrations are not normally found in human plasma[19], it could contribute significantly to effects observed in vitro in whole tissue preparations.

These findings demonstrate that prostaglandin catabolism is subject to inhibition by indomethacin in vitro and that the first three enzymes in the complex catabolic pathway have different sensitivities to this drug. In vivo a similar blockade may occur because the concentration range required to block catabolism in vitro (at least for 9-PGDH and 13-PGR) lies within the range found in human plasma after administration of this drug[19]. Because a multiplicity of steps exist in the catabolism of prostaglandins and blockade by this drug can take place at several of these steps, the complete profile of catabolites

[1] J. R. VANE, Nature New Biol. *231*, 232 (1971).
[2] J. B. SMITH and A. L. WILLIS, Nature New Biol. *231*, 235 (1971).
[3] S. H. FERREIRA, S. MONCADA and J. R. VANE, Nature New Biol. *231*, 237 (1971).
[4] M. HAMBERG, Biochem. Biophys. Res. Commun. *49*, 720 (1972).
[5] J. G. COLLIER and R. J. FLOWER, Lancet *2*, 852 (1971).
[6] J. R. VANE and K. I. WILLIAMS, Br. J. Pharmac. *45*, 146 P (1972).
[7] P. DAVISON, P. W. RAMWELL and A. L. WILLIS, Br. J. Pharmac. *46*, 547 P (1972).
[8] P. HEDQVIST, L. STJÄRNE and Å. WENNMALM, Acta physiol. scand. *83*, 430 (1971).
[9] B. SAMUELSSON and Å. WENNMALM, Acta physiol. scand. *83*, 163 (1971).
[10] P. H. CHANH, M. JUNSTAD and Å. WENNMALM, Acta physiol. scand. *86*, 563 (1972).
[11] A. J. LONIGRO, H. D. ITSKOVITZ, K. CROWSHAW and J. C. MCGIFF, Circulation Res. *32*, 712 (1973).
[12] K. HERBACZYNSKA-CEDRO and J. R. VANE, Adv. Biosciences *9*, 45 (1972).
[13] H. A. DAVIS and E. W. HORTON, Br. J. Pharmac. *46*, 658 (1972).
[14] J. W. AIKEN, Nature, Lond. *240*, 21 (1972).
[15] P. B. MARLEY, Prostaglandins *4*, 251 (1973).
[16] R. CHESTER, M. DUKES, S. R. SLATER and A. L. WALPOLE, Nature, Lond. *240*, 37 (1972).
[17] C. PACE-ASCIAK and D. MILLER, Experientia *30*, 590 (1974).
[18] C. PACE-ASCIAK and D. MILLER, Prostaglandins *4*, 391 (1973).
[19] R. FLOWER, R. GRYGLEWSKI, K. HERBACZYNSKA-CEDRO and J. R. VANE, Nature New Biol. *238*, 104 (1972).

ought to be measured in biological fluids rather than the major urinary metabolite alone before conclusions can be drawn whether a drug inhibits only prostaglandin biosynthesis or catabolism or both. We are presently exploring this more complete approach towards the effect of drugs on the PG system with several other compounds which we have found to inhibit prostaglandin catabolism[20,21].

[20] We thank Drs. J. E. PIKE and U. AXEN, The Upjohn Co., Kalamazoo, U.S.A. for their generous supplies of prostaglandins.
[21] Supported by a grant No. MA-4181 to C.P.A. from the Medical Research Council of Canada.

Zusammenfassung. Es zeigt sich, dass jedes der 3 Enzyme, die den Abbau von Prostaglandin in den Nieren erwachsener Ratten verursachen, durch schwache Konzentrationen von Indomethacin in steigendem Ausmass inhibiert werden können: 9-PGDH > 13-PGR > 15-PGDH.

C. PACE-ASCIAK and S. COLE

Research Institute, The Hospital for Sick Children, 555 University Avenue, Toronto M5G 1X8 (Canada), 9 September 1974.

Identification of Precoccinellin in the Ladybird Beetle, *Coleomegilla maculata*[1-3]

Coccinellid beetles have long been known to possess defensive compounds associated with 'reflex bleeding'[4], and the investigation of several European lady bugs has yielded the structures of the specific alkaloids[5-7]. Likewise, during an investigation of the volatiles of the lady beetle, *Coleomegilla maculata*, we have isolated and identified a defensive alkaloid, precoccinellin.

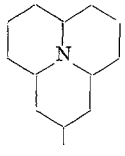
Isolation procedures. The beetles were steam-distilled for 2 h in an all-glass system, and the distillate was extracted with methylene chloride. The extract was concentrated in vacuo and chromatographed by GLC on a 20 ft, 1/8 inch O.D. stainless steel column packed with 10% SE-30. Column temperature was 160 °C, and carrier gas pressure at the inlet was 60 ψ. Retention time of the alkaloid was 10 min. I_k = 1475. The compound was collected from a stream splitter attachment on the gas chromatograph. *IR-spectrum.* The IR-spectrum in CCl_4 included ν_{max} 1020, 1040, 1120, 1130, 1325, 1385, 1450, 2775, 2860, 2925, 2950 CM^{-1}.

PMR-spectrum. The PMR-spectrum in CCl_4 showed ppm (δ) 0.89, 1.24, 1.38, 1.55, 1.64.

Mass spectrum m/e. 41 (100), 192 (66), 150 (51), 151 (47), 55 (47), 42 (44), 137 (34), 136 (32), 164 (28), 67 (26), 122 (25), 93 (22), 82 (19), 178 (18), 108 (15), 96 (13); M^+ = 193. Essentially the same as the precoccinellin isolated by TURSCH et al.[5].

Reactions. Hydrogenation (Pd on charcoal; hydrogen under pressure) – no effect. Lithium aluminum hydride reduction – no effect. NaOH treatment – no effect. HCl treatment – hydrochloride formed.

Based on these experiments and on data presented by TURSCH et al.[6], we propose that the compound isolated from *C. maculata* is precoccinellin:



(Dodecahydro-2-methylpyrido[2,1,6-*de*]quinolizine)

Extraction of whole beetles with methanol revealed no additional alkaloids. The compound is bitter to the taste, and it is assumed that it performs in a defensive mode as do other similar coccinellid alkaloids.

Zusammenfassung. Isolierung und Strukturzuteilung für ein Alkaloid aus *Coleomegilla maculata* (Coleoptera: Curculionidae).

R. D. HENSON[8], A. C. THOMPSON[8], P.A. HEDIN[8], P. R. NICHOLS[9] and W. W. NEEL[9]

United States Department of Agriculture, Agricultural Research Service, Boll Weevil Research Laboratory, P. O. Box 5367, Mississippi State (Mississippi 39762, USA), 1 August 1974.

[1] Coleoptera: Curculionidae.
[2] In cooperation with the Mississippi Agricultural and Forestry Experiment Station, Mississippi State, Mississippi 39762. Received for publication.
[3] Mention of a proprietary product does not necessarily imply endorsement of this product by the USDA.
[4] R. M. HAPP and T. EISNER, Science *134*, 329 (1961).
[5] B. TURŠCH, D. DALOZE, M. DUPONT, J. M. PASTEELS and MARIE-CLAIRE TRICOT, Experientia *27*, 1380 (1971).
[6] B. TURSCH, D. DALOZE, M. DUPONT, C. HOUTELE, M. KAISIN, J. M. PASTEELS, and D. ZIMMERMANN, Chimia *25*, 307 (1971).
[7] R. KARLSSON and D. LOSMAN, J. chem. Soc., Chem. Commun. *11*, 626 (1972).
[8] Boll Weevil Research Laboratory, USDA, ARS, Mississippi State, Mississippi 39762, USA.
[9] Department of Entomology, Mississippi State University, Mississippi State, Mississippi 39762, USA.

Microheterogeneity of Staphylococcal Enterotoxin C_2

Staphylococcal enterotoxin C_2 belongs to a closely related group of simple proteins that are causative agents of staphylococcal food poisoning. The toxin has been purified to homogeneity, and several of its physical-chemical properties have been determined[1]. We also isolated enterotoxin C_2 in a homogeneous form as judged by gel filtration, immunodiffusion, ultracentrifugation, and N-terminal amino acid analysis (to be published elsewhere). However, disc-gel electrophoresis showed 2 bands that formed an immunoprecipitate with specific