electrophilic center at the binding site of the enzyme. An alternative to these explanations is that the observation of the lower Michaelis constants for the halogen-substituted dipeptides may depend in some way on the electron-withdrawing inductive effect of the halogen atoms on the aromatic ring of the tyrosine nucleus. In this regard the powerful influence of the bromo and iodo substituents is evidenced by the pK_a' OH values measured for the substituted phenolic groups as shown in Table I. The pK_a' OH values for the hydroxyl groups of the tyrosine residues in N-acetylphenylalanyl-L-3,5-dibromotyrosine and N-acetylphenylalanyl-L-3,5-diiodotyrosine are much lower than that in the unsubstituted dipeptide.

Another point which requires explanation is that in a recent report of work on the pepsin-catalyzed hydrolysis of N-acetyl-L-phenylalanyl-L-diiodotyrosine, inhibition by the product, N-acetyl-L-phenylalanine, was not found[6] despite the observation of competitive inhibition by the same product in studies on the hydrolysis of N-acetyl-L-phenylalanyl-L-tyrosine[3] (see Table I). In our investigation of the hydrolysis of N-acetyl-L-phenylalanyl-L-3,5-dibromotyrosine, we found inhibition by N-acetyl-L-phenylalanine although the value of K_I which we measured cannot be compared directly to that observed in the hydrolysis of N-acetyl-L-phenylalanyl-L-tyrosine since Silver, *et al.*,[3] did not report a value for K_I.

In summary, our results indicate that the hydrolytic behavior of the dibromo dipeptide lies in between that observed for the diiodo dipeptide and that of the substituted dipeptide. In order to further elaborate on the causes of trends in both K_m and k_{cat}, we are systematically varying the polarizability and size of substituents. For these reasons the kinetics of the pepsin-catalyzed hydrolysis of N-acetyl-L-phenylalanyl-L-3,5-dinitrotyrosine and related dipeptides are now under study.

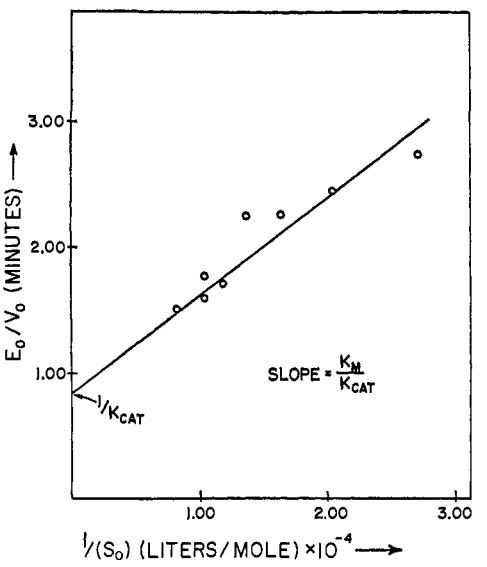


Figure 1. Lineweaver–Burk plot for the hydrolysis of N-acetyl-L-phenylalanyl-L-3,5-dibromotyrosine by 7×10^{-7} *M* pepsin at pH 2.0.

Communications to the Editor

A Total Synthesis of a Natural Prostaglandin

Sir:

Prostaglandins, a family of C_{20} prostanoic acids of the same carbon skeleton[1] first isolated by von Euler[2] and Goldblatt[3] as crude extracts from sheep seminal vesicular glands or human seminal plasma, are now known to be physiologically important lipids[1,4-6] widely distributed in nature.[1,7] In an elegant series of chemical, spectral, and crystallographic studies Bergstrom and co-workers first isolated PGE_1 (prostaglandin E_1), (15*R*)-9-oxo-11α,15-dihydroxyprost-13-*trans*-enoic acid,[1c] in pure form[8] and established its structure (I).[1a,b,9,10] We now report the synthesis (see Scheme I) of the racemic ethyl ester of (15*R*)-9-oxo-11α,15-dihydroxyprostanoic acid (dihydro-PGE_1, XIVa), a biologically potent[11,12] naturally occurring[13] metabolite of PGE_1.

Formylation (sodium hydride–ethyl formate) of 3-ethoxy-2-cyclopentenone (II)[14] afforded nearly quantitative yields of the sodium salt of the 5-hydroxymethylene derivative III. Heterogeneous reaction of III in chloroform with ethyl bromoacetate and triphenylphosphine gave IV[15] in 70% yield, mp 64–65°, λ_{max}^{EtOH} 251 (ϵ 14,500) and 260 mμ (ϵ 14,300), prepared also, after neutralization of III (although less conveniently), with carbethoxymethylenetriphenylphosphorane.

Acid hydrolysis of IV yielded the enol Va[15] [mp 155–160°, λ_{max}^{EtOH} 275.5 mμ (ϵ 17,900)] which, after treatment

(1) For leading references and nomenclature see: (a) S. Bergström and B. Samuelsson, *Ann. Rev. Biochem.*, **34**, 101 (1965); (b) B. Samuelsson, *Angew. Chem. Intern. Ed. Engl.*, 4, 410 (1965); (c) B. Samuelsson, *J. Biol. Chem.*, **238**, 3229 (1963); (d) E. W. Horton, *Experientia*, **21**, 113 (1965).

(2) U. S. von Euler, *Arch. Exptl. Pathol. Pharmakol.*, **175**, 78 (1934).

(3) M. W. Goldblatt, *J. Soc. Chem. Ind.* (London), **52**, 1056 (1933); *J. Physiol.* (London), **84**, 208 (1935).

(4) P. W. Ramwell, J. E. Shaw, and J. Kucharski, *Science*, **149**, 1390 (1965).

(5) G. L. Avanzino, P. B. Bradley, and J. H. Wolstencroft, *Nature*, **209**, 87 (1966).

(6) M. Bygdeman, *Acta Physiol. Scand. Suppl.*, **63**, 242 (1964).

(7) B. Samuelsson, *Proc. Intern. Congr. Endocrinol., 2nd, London, 1964*, 847 (1965).

(8) S. Bergström and J. Sjöval, *Acta Chem. Scand.*, 14, 1701 (1960).

(9) S. Bergström, R. Ryhage, B. Samuelsson, and J. Sjöval, *ibid.*, **16**, 501 (1962).

(10) (a) S. Abrahamson, S. Bergström, and B. Samuelsson, *Proc. Chem. Soc.*, 332 (1962); (b) S. Abrahamson, *Acta Cryst.*, **16**, 409 (1963).

(11) E. Ånggård, *Acta Physiol. Scand.*, in press.

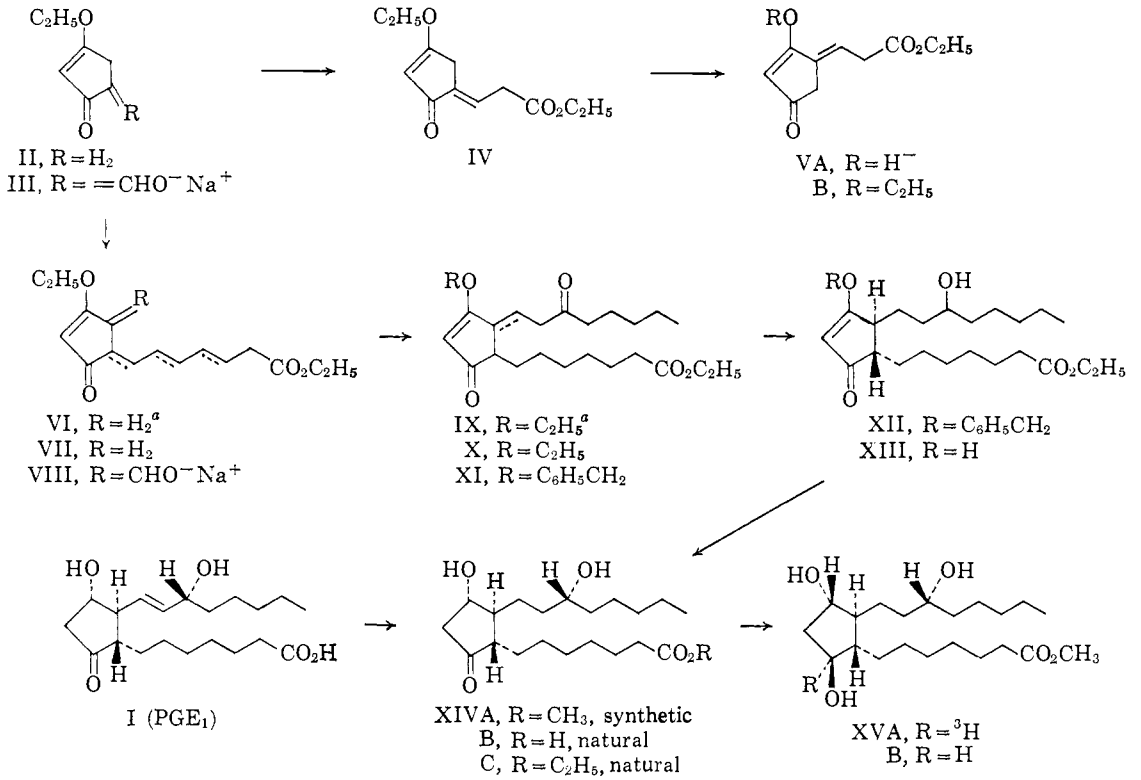
(12) J. Weeks, unpublished observations.

(13) E. Ånggård and B. Samuelsson, *J. Biol. Chem.*, **239**, 4097 (1964).

(14) H. O. House and G. H. Rasmusson, *J. Org. Chem.*, **28**, 27 (1963).

(15) Analysis within journal requirements and compatible nmr spectra.

Scheme I



[a] In compounds VI and IX, the side chain is unsaturated (dotted bonds). VII, VIII, X, and XI have saturated side chains.

with diazoethane, gave Vb[15] [mp 48–50°; λ_{max}^{EtOH} 206 (ϵ 12,400), 211 (ϵ 11,400), and 269.5 mμ (ϵ 16,600)]. Ultraviolet maxima of IV and Vb confirm the structures assigned and hence the structure of III.

In a key extension of the Wittig reaction, addition of III to a solution of ethyl 6-bromosorbate[16] and triphenylphosphine yielded VI[15] [mp 85–88°, λ_{max}^{EtOH} 216 (ϵ 10,750), 252 (ϵ 8650), and 327.5 mμ (ϵ 38,950)] in 35–50% yield. Catalytic hydrogenation selectively reduced the side chain to give VII.[15] Again, formylation (sodium hydride–ethyl formate) produced the sodium salt of the hydroxymethylene derivative VIII which reacted with *n*-hexanoylmethylenetriphenylphosphonium chloride[17] in chloroform to give IX[15] as a mixture of geometric isomers (48% yield). Hydrogenation of the exocyclic bond (5% palladium–charcoal; trace of triethylamine) proceeded in 85% yield to give X.[15,18]

Acid-catalyzed solvolysis of X in benzyl alcohol gave XI[15] and the isomeric enol ether. Without separation, XI was reduced with lithium tri-*t*-butoxyaluminum hydride to the corresponding 15-hydroxy compound XII[15,19] in 85% yield. Catalytic hydrogenolysis of this mixture (5% palladium–charcoal) gave the β-diketone XIII which was subjected to prolonged catalytic hydrogenation (30% rhodium–charcoal) to produce a complex mixture. A major component of this mixture, XIVa,[15] obtained in 11% yield as a fraction homogeneous on tlc, exhibited a polarity identical with that of authentic dihydro-PGE$_1$ ethyl ester (XIVc).

Although both materials were oils, XIVa was shown both to have the correct gross structure and to include material of proper stereochemistry in the following manner.[20]

Both compounds exhibited the same mobility on tlc, possessed identical infrared spectra, underwent alkaline isomerization[1b,21] to products (λ_{max} near 240 mμ) with the same mobility on tlc, had common nmr maxima with no extraneous peaks, showed the same parent ion at *m/e* 384 and prominent fragments at *m/e* 366, 348, 295, 210, and 192 in the mass spectrum,[22,23] exhibited muscle-stimulating and vasodepressor activity in the guinea pig ileum and pentolinium-blocked dog assays[24,25] of the same order of magnitude,[12] and were dehydrogenated enzymatically by the 15-dehydrogenase from pig lung.[11,26,27]

Final confirmation of structure and stereochemistry of a component of the final mixture was obtained by radioisotope dilution. Authentic crystalline methyl

(16) H. E. Ungnade and T. R. Hopkins, *J. Am. Chem. Soc.*, 73, 3091 (1951).

(17) S. Trippett and D. M. Walker, *J. Chem. Soc.*, 1266 (1961).

(18) Isomerization of the Δ^{12} to the less-hindered Δ^{13} bond is believed to occur during reduction of IX to X in the presence of base. Although X contains two asymmetric centers, it is largely one racemate with *trans* substituents, homogeneous by tlc and with a well-defined nmr spectrum. Mass spectral and labeling studies confirm this stereochemical preference.

(19) This compound is undoubtedly a mixture of diastereoisomers as little stereochemical control appears likely here.

(20) As this material occasionally decomposed on standing, the final product was freshly purified before most of the following comparisons were made.

(21) S. Bergström, R. Ryhage, B. Samuelsson, and J. Sjövall, *J. Biol. Chem.*, 238, 3555 (1963).

(22) Especially significant were peaks at *m/e* 210 and 192 formed by loss of the acid side chain through McClafferty rearrangement: F. W. McClafferty, *Anal. Chem.*, 31, 82, 1959.

(23) The similarity of the mass spectra is considered to support the *trans* side chain configuration since *cis*- and *trans*-dialkylcyclopentanes have been reported to give different mass spectra: J. Momigny and P. Natalis, *Bull. Soc. Chim. Belges*, 66, 26 (1957).

(24) E. E. Muirhead, E. G. Daniels, E. Booth, W. A. Freyburger, and J. W. Hinman, *Arch. Pathol.*, 80, 43 (1965).

(25) E. G. Daniels, J. W. Hinman, B. A. Johnson, F. P. Kupiecki, J. W. Nelson, and J. E. Pike, *Biochem. Biophys. Res. Commun.*, 21, 413 (1965).

(26) S. Bergström and B. Samuelsson, private communication.

(27) E. Änggård and B. Samuelsson, *Arkiv Kemi*, 25, 293 (1966).

(15*R*)-9β,11α,15-trihydroxyprostanoate (dihydro-$PGF_{1\beta}$ methyl ester, XVb),[15] mp 62–63°, was prepared from PGE_1 (I) by catalytic hydrogenation[9,28] and reduction with sodium borohydride.[29]

The synthetic racemic ketoester, XIVa, was reduced with sodium borotritide, hydrolyzed, and reesterified with diazomethane, yielding a mixture of 9-tritiated-9-hydroxy epimers. A sample (XVa) enriched in the 9β-hydroxy isomer by chromatography on silica gel was combined with optically active, crystalline XVb. Two crystallizations afforded material of constant melting point (62–63°) and specific activity (0.249 μcurie/mg) unchanged on two further crystallizations (0.238 and 0.236 μcurie/mg). Isotope dilution indicated that XIVa contained at least 22% of product of the same optical configuration at all asymmetric centers as XIVc.[30,31]

Acknowledgment. The authors wish to express their appreciation to their associates in these laboratories, especially Marvin Grostic for his contributions in mass spectroscopy, Byron Johnson for preparation of intermediates, and George Cartland and James Weeks for biological studies.

(28) B. Samuelsson and G. Stallberg, *Acta Chem. Scand.*, **17**, 810 (1963).

(29) S. Bergström, L. Krabisch, B. Samuelsson, and J. Sjövall, *ibid.*, **16**, 969 (1962).

(30) Stereochemical randomness would produce only 6% of the natural isomer in the introduction of four asymmetric centers.

(31) NOTE ADDED IN PROOF. D. H. Nugteren and D. A. vanDorp, Unilever Laboratories, and B. Samuelsson and S. Bergstrom, Karolinska Institute, announced independently at the 2nd Nobel Symposium on The Prostaglandins, June 6, 1966, that reexamination of the original data indicates that the absolute configuration of the prostaglandins should now be represented by the mirror image of that used in this paper and in earlier publications.

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Research Laboratories, The Upjohn Company
Kalamazoo, Michigan
Received May 27, 1966

Ground- and Transition-State Free Energy Relationships in σ and π Routes to the Nonclassical 7-Norbornenyl Cation[1]

Sir:

One of the most interesting of the nonclassical carbonium ions is the intermediate II in solvolysis of *anti*-7-norbornenyl derivatives[2] such as I-OTs. In this communication we report on kinetic and thermodynamic product control and ground- and transition-state free energy relationships in σ and π routes[3] to cation II in methanolysis.

A tricyclic derivative suitable for the σ route to cation II, namely, III-OCH_3, was obtained very recently in an elegant investigation by Tanida, Tsuji, and Irie,[4a] who observed considerable tricyclic product IV-OCH_3 in methanolysis of 7-norbornadienyl chloride in the presence of substantial concentrations of sodium methoxide. Hydrogenation of IV-OCH_3 led to III-OCH_3, an 85% pure sample of which was isolated. Tanida and co-workers[4a] also reported tricyclic III-OCH_3 from methanolysis of I-OTs with alkali, but in only 1% yield.

(1) Research supported by the National Science Foundation.

(2) *E.g.*, (a) S. Winstein, A. H. Lewin, and K. C. Pande, *J. Am. Chem. Soc.*, **85**, 2324 (1963); (b) H. C. Brown and M. Bell., *ibid.*, **85**, 2324 (1963).

(3) S. Winstein and P. Carter, *ibid.*, **83**, 4485 (1961).

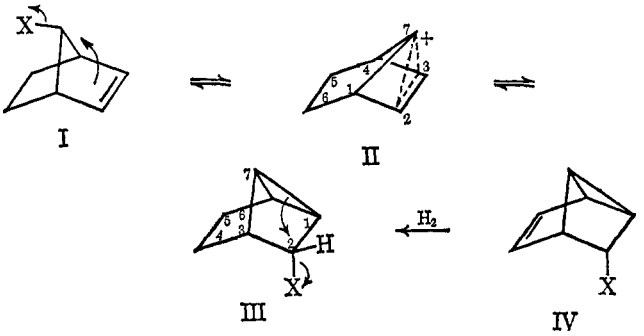
(4) (a) H. Tanida, T. Tsuji, and T. Irie, *ibid.*, **88**, 864 (1966); (b) H. Tanida and Y. Hata, *J. Org. Chem.*, **30**, 977 (1965).

Table I. Kinetic Product Control in Methanolysis of I-OTs[a] at 25.0°

$[NaOCH_3]$, *M*	% I-OCH_3	% III-OCH_3
...[b]	100	...
3.5×10^{-8}[c]	99.7	0.3[d]
0.20	93.6	6.4
0.88	79.7	20.3
1.93	65.3	34.7
3.98	48.5	51.5

[a] *Ca.* 0.01 or 0.02 *M* I-OTs. [b] Solution allowed to become acidic. [c] 0.0452 *M* NaOAc-0.0394 *M* AcOH buffer; (MeO^-) varies from 7.5×10^{-8} *M* to 3.8×10^{-8} *M*. [d] This material disappears in acidic 80% acetone with the same half-life as for III-OCH_3.

We noted and remarked previously[2a] on the fact that the $C_7:C_2$ reactivity ratio in cation II is quite dependent on the nature of the nucleophile when it is varied widely. Thus, tricyclic alcohol III-OH was not observed[2a] from neutral hydrolysis of I-OTs, but considerable tricyclic hydrocarbon III-H is obtained from borohydride trapping,[2] and some tricyclic nitrile III-CN is formed in the reaction of I-Cl with NaCN in aqueous alcohol.[4b] We have now observed striking changes in product composition in methanolysis of I-OTs as methoxide concentration is varied. In methanol at 25.0°, I-OTs undergoes first-order methanolysis with a rate constant of 1.31×10^{-3} sec^{-1} to form I-OCH_3 quantitatively. No tricyclic III-OCH_3 is observed, but even if it were formed, it would not survive the acidic conditions since it is very easily isomerized to I-OCH_3 in the presence of acid. As summarized in Table I, methanolysis of I-OTs with increasing concentrations of $NaOCH_3$ gives increasing proportions of tricyclic III-OCH_3 in the product. Thus, with 4 *M* $NaOCH_3$, the observed ether contains 51.5% tricyclic III-OCH_3. The data make it evident that sodium methoxide does not serve merely to preserve the tricyclic III-OCH_3, but instead is largely responsible for its formation.



The kinetic control product from neutral methanolysis of I-OTs was determined from solvolysis in methanol containing an AcOH–NaOAc buffer. Control experiments showed the tricyclic III-OCH_3 to be fully stable under these conditions. The product ether from buffered methanolysis contained 0.3% of the tricyclic III-OCH_3, as determined by vpc. Examination of the nmr spectrum, using a Varian C-1024 time-averaging computer (CAT), confirmed the presence of III-OCH_3 with its characteristic C_2 α-proton quartet centered at τ 6.35. Thus, kinetic product control in methanolysis of I-OTs involves a $C_7:C_2$ reactivity ratio[5] of *ca.* 300.

(5) The situation regarding kinetic and thermodynamic control in