lar reaction mechanism may occur for wheat carboxypeptidase. The rate constant for deacylation may be made small by the presence of proline in the penultimate position from the carboxyl terminus of substrates. In wheat carboxypeptidase, the nature of the amino acid in the penultimate position from the carboxyl terminus of the substrate generally appears to be subjected to the influence of the rate constant for deacylation.

In conclusion, the K_m value of wheat carboxypeptidase was not appreciably influenced by the structure of the substrate. However, the nature of the penultimate amino acid from the carboxyl terminus of the substrate dramatically changed the k_{cat} value.

EXPERIMENTAL

Materials. *N*-Acyl dipeptide, Z–Gly–Pro–Leu–Gly, angiotensin II and bradykinin were purchased from the Protein Research Foundation, Osaka.

Carboxypeptidase from wheat. Wheat bran from bread and common wheat (*Triticum aestivum* L.) was purchased from Nissin Seifun Co. Ltd., Tokyo. The crystalline wheat carboxypeptidase from wheat bran was prepared acccrding to ref. [1]. The enzyme used here was homogeneous on disc electrophoresis at pH 4 and on analytical ultracentrifugation.

Kinetic studies. The values of K_m and k_{cat} were determined graphically from Lineweaver–Burk plots. When *N*-acyl dipeptides were used as substrates, the released amino acids were determined by the ninhydrin method. The initial rates of hydrolysis of Z–Gly–Pro–Leu–Gly, angiotensin II and bradykinin were determined with an automatic amino acid analyser, Hitachi model 835-30. No amino acids other than the carboxyl-terminal amino acid from the substrate used in the kinetic study were observed.

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AN ACETYLENIC TRIOL FROM *HYOSERIS LUCIDA**

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Key Word Index—*Hyoseris lucida*; Compositae; acetylenic compound; C_{14}-enediyne.

Abstract—The aerial parts of *Hyoseris lucida* afforded a new acetylenic triol. The structure was elucidated by spectroscopic methods and a few chemical transformations.

So far little is known on the chemistry of the small genus *Hyoseris* (tribe Cichorieae). A preliminary investigation of the roots of *H. lucida* L. gave no characteristic compounds [1]. However, from the aerial parts, we have now isolated a crystalline compound, molecular formula $C_{14}H_{20}O_3$, which was covered rapidly with a deep blue coloured polymer when exposed to light. This was an indication that an acetylenic compound may be present. The characteristic UV maxima of an enediyne [2] established this proposal. The ^{1}H NMR spectrum (Table 1) showed the typical signals of a *trans*-configurated propenyl end group and four lowfield signals (δ 3.69 and 3.75 *dt*, 3.59 and 3.47 *dddd*) indicated the presence of a primary and two secondary hydroxyl groups. A broadened triplet at δ 2.50 was obviously due to a methylene group adjacent to the triple bonds. Spin decoupling showed that the secondary hydroxyl groups were at vicinal carbons while the signals of the primary hydroxyl group were coupled with a multiplet at δ 1.74. These results required a C_{14}-enediyne triol since the secondary hydroxyl groups could be placed

*Part 264 in the series "Polyacetylenic Compounds". For Part 263 see Bohlmann, F. and Ahmed, M. (1982) *Phytochemistry* **21**, 2742.

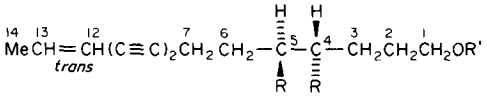
Table 1. ^{1}H NMR spectral data of compounds 1–3 (400 MHz, $CDCl_3$, TMS as int. standard)

	1	2	3*
H-1	3.69 *dt*	4.11 *t*	4.08 *dt*
H-1′	3.75 *dt*		4.12 *dt*
H-2, H-3	1.74, 1.58 *m*	1.71, 1.55 *m*	1.73, 1.59 *m*
H-4	3.59 *m*	3.59 *dddd*	3.68 *dt*
H-5	3.47 *m*	3.46 *dddd*	3.64 *dt*
H-6	1.74, 1.58 *m*	1.71 *m*	1.73 *m*
H-7	2.50 *br t*	2.50 *br t*	2.53 *br ddd* 2.46 *br ddd*
H-12	5.50 *dqt*	5.50 *dqt*	5.50 *dqt*
H-13	6.29 *dq*	6.29 *dq*	6.29 *dq*
H-14	1.79 *dd*	1.79 *dd*	1.78 *dd*
OH	2.40 *br s*	2.14 *br d* 2.08 *br d*	—
OAc		2.04 *s*	2.05 *s*

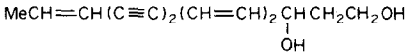
*Acetonide-Me 1.33 *s*, 1.34 *s*.

J (Hz): 1, 1′ ~ 12; 1, 2 ~ 7; 3, 4 = 5, 6 ~ 8; 3′, 4 = 5′, 6 = 4; 4, 5 = 8.5; 4, OH = 5, OH = 5; 6, 7 = 7.5; 7, 12 = 1; 12, 14 = 1.5; 12, 13 = 16; 13, 14 = 7; compound **2**: 1, 2 = 7; compound **3**: 4, 5 = 4; 6, 7 = 8; 7, 7′ = 17.

only at C-3 and C-4 or C-4 and C-5. As, however, the signals of the methylene groups were overlapped multiplets a clear decision was not possible. Partial acetylation led to a monoacetate and its ^{1}H NMR spectrum (Table 1) supported the assumptions but again showed overlapped multiplets for the methylene groups. Also the ^{1}H NMR spectrum (Table 1) of the corresponding acetonide did not allow a clear assignment of the position of the secondary oxygen functions. However, the vicinal coupling of the protons under the oxygens indicated a *trans*-orientation. The mass spectrum of the acetonide strongly supported the presence of a 4,5-acetonide, especially the fragment *m/z* 217 ($C_{14}H_{17}O_2$) which could be explained best as a loss of $(CH_2)_3OAc$. Several further fragments agreed well



1 R=OH, R′=H
2 R=OH, R′=Ac
3 R=O,O, R′=Ac



4

with the proposed structure **3** for the acetonide. Thus the natural triol was 1,4,5-trihydroxytetradec-12*t*-en-8,10-diyne.

From *H. radiata* L. we have isolated the known C_{14}-endiynediene **4** [3]. So far only a few acetylenes have been reported from representatives of the Cichorieae [4], most of them are C_{10} compounds. Further investigations are necessary to see whether the acetylenic compounds are of chemotaxonomic interest.

EXPERIMENTAL

The plant material of *H. lucida* was collected in early March 1981 from Borg-El-Arab, 50 km W. of Alexandria. The plant was previously authenticated by the Late Professor Dr. V. Täckholm, Faculty of Science, Cairo University. A voucher specimen is deposited in the Department of Pharmacognosy, Faculty of Pharmacy, University of Alexandria. Fresh aerial parts (500 g) were extracted with $CHCl_3$ and the extract was separated first by CC (Si gel). The fractions obtained with $CHCl_3$–MeOH (49 : 1) were induced to crystallize, finally 10 mg **1** was obtained.

Tetradeca-12t-ene-8,10-diyne-1,4,5-triol (**1**). Colourless crystals, mp 110–112° ($CHCl_3$–EtOAc) which rapidly turned blue; UV λ_{max}^{MeOH} nm (ε): 282 (11 200), 266 (13 800), 251 (9800), 239 (7100), 226 (5000), 212 (37 400); IR ν_{max}^{KBr} cm^{-1}: 3350, 3470 (OH), 2240, 2150 (C≡C); MS *m/z* (rel. int.): 236.141 $[M]^+$ (7) ($C_{14}H_{20}O_3$ required 236.141), 218 $[M-H_2O]^+$ (3), 203 $[218-Me]^+$ (24), 147 $[M-CH(OH)CH_2CH_2CH_2OH]^+$ (19), 117 $[C_9H_9]^+$ (44), 89 $[C_4H_9O_2]^+$ (23), 71 $[89-H_2O]^+$ (100).

2 mg **1** were dissolved in 0.1 ml Ac_2O. After 1 hr at 22° evaporation afforded, after TLC (Et_2O), 1.5 mg **2**, which was dissolved in Me_2CO which containing 3 mg *p*-toluene sulfonic acid. After 12 hr, TLC (Et_2O–petrol, 1 : 1) gave 1 mg **3**, colourless gum, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 2250 (C≡C), 1750, 1250 (OAc); MS *m/z* (rel. int.): 318.183 $[M]^+$ (9) ($C_{19}H_{26}O_4$, required 318.183), 303 $[M-Me]^+$ (18), 259 $[M-OAc]^+$ (6), 258 $[M-HOAc]^+$ (3), 217.123 $[M-(CH_2)_3OAc]^+$ (11) ($C_{14}H_{17}O_2$), 159 $[217-Me_2CO]^+$ (44), 129 $[159-CH_2O]^+$ (70), 103 $[129-C_2H_2]^+$ (58), 77 $(103-C_2H_2]^+$ (100).

H. radiata (*voucher* 79/1376). Grown from seeds from the Botanic Garden, Marburg, West Germany. The roots (50 g) afforded 3 mg **4**, while the aerial parts (250 g) afforded 20 mg lupeylacetate and 10 mg taraxasterylacetate.

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