2 Yokota, T., Arima, M., and Takahashi, N., Tetrahedron Lett. *23* (1982) 1275.
3 Tompson, M.J., Mandava, N.B., Meudt, W.J., Lusby, W.R., and Spaulding, D.W., Steroids *38* (1981) 567.
4 Takatsuto, S., Ying, B., Morisaki, M., and Ikekawa, N., Chem. Pharm. Bull. *29* (1981) 903. Brassinone (**IV**), m.p.254–255 °C (from ethyl acetate) was obtained by deprotection of the intermediate to 28-norbrassinolide (**III**).
5 Takatsuto, S., and Ikekawa, N., Chem. Pharm. Bull. *30* (1982) in press.
6 Marumo, S., Hattori, H., Abe, H., Nonoyama, Y., and Munakata, K., Agric. Biol. Chem. *32* (1968) 528.
7 Wada, K., Marumo, S., Ikekawa, N., Morisaki, M., and Mori, K., Pl. Cell Physiol. *22* (1981) 323.
8 Ikekawa, N., Takatsuto, S., Marumo, S., Abe, H., Morishita, T., Uchiyama, M., Ikeda, M., Sassa, T., and Kitsuwa, T., Proc. Japan Acad. B *59* (1983) 101.
9 Abe, H., Morishita, T., Uchiyama, M., Marumo, S., Munakata, K., Takatsuto, S., and Ikekawa, N., Agric. Biol. Chem. *46* (1982) 2609.
10 Morishita, T., Abe, H., Uchiyama, M., Marumo, S., Takatsuto, S., and Ikekawa, N., Phytochemistry *22* (1983) in press.
11 Ikeda, M., Nukina, M., Sassa, T., Takatsuto, S., and Ikekawa, N., Agric. Biol. Chem. *47* (1983) in press.
12 Takatsuto, S., Ying, B., Morisaki, M., and Ikekawa, N., J. Chromatography *239* (1982) 233.
13 Ishiguro, M., Takatsuto, S., Morisaki, M., and Ikekawa, N., J. chem. Soc. chem. Commun. *1980*, 962.
14 Takatsuto, S., Yazawa, N., Ikekawa, N., Morishita, T., and Abe, H., Phytochemistry *22* (1983) in press.

0014-4754/83/040351-03$1.50+0.20/0

Synthesis and biological activities of [β-Malyl^1]- and [β-Malyl^1, Leu^8]-angiotensin II analogues[1]

P. Cordopatis, J. Matsoukas, A. Michel, J. Janen and D. Theodoropoulos[2,3]

Laboratory of Organic Chemistry, University of Patras, Patras (Greece), and Laboratory of Biological Chemistry, University of Mons, Mons (Belgium), August 24, 1982

Summary. The synthesis of [β-Malyl^1]- and [β-Malyl^1, Leu^8]-angiotensin II using a solid phase procedure is reported. The replacement of the N-terminal amino group of aspartic acid by a hydroxyl group gives analogues with lower affinity than [Asn^1]- and [Asn^1, Leu^8]-AII. However, the isoster [β-Malyl^1]-AII shows higher potency than [Asn^1]-AII and this may be due to metabolic or enzymatic resistance.

We wish to describe the syntheses of the agonist and the [Leu^8]-antagonist isosters of angiotensin II, in which the N-terminal primary amino group was replaced by a hydroxyl group. These isosters, [β-Malyl^1]- and [β-Malyl^1, Leu^8]-AII contain the α-hydroxy-analogue of L-aspartic acid at position 1. The L-malic acid was bound via its β-carboxyl group instead of its α because [β-Asp^1]-AII is well known to exhibit higher potency than the natural hormone[4,5]. The pharmacological activities of these analogues were investigated to provide further information about the importance of the N-terminus amino group and the basicity of its nitrogen for maximum agonistic and antagonistic propterties.

Methods. Firstly, the synthesis of α-benzyl-L-malate was achieved by 2 independent routes, and its structure was confirmed by ^{1}H NMR and other physical techniques. L-Malic acid, when treated with benzyl bromide in the presence of triethylamine, gave dibenzyl-L-malate (**I**). The homogeneous oily product was tritylated in the presence of pyridine and the dibenzyl-O-trityl-L-malate (**II**) obtained, owing to the well known steric hindrance of the trityl group[6], was selectively saponified with 2N LiOH (2:1 molar ratio). The resulting α-benzyl-O-trityl-L-malate (**III**) was isolated as the dicyclohexylammonium salt (**IV**) in crystalline form. On the other hand, the disilver salt of L-malic acid was treated with thionyl chloride to give the malic anhydride[7], which, without purification, was further treated with benzyl alcohol. The α-benzyl-L-malate (**V**) thus obtained was isolated as the dicyclohexylammonium salt (**VI**) and its structure was confirmed as follows. Compound **VI**, after being treated with 5% citric acid, washing with water and drying, was tritylated in the presence of pyridine. The obtained α-benzyl-O-trityl-L-malate and the corresponding dicyclohexylammonium salt, were proved to be identical with those obtained by the former route (table 1). The ^{1}H NMR-data of α-benzyl-O-trityl-L-malate (obtained by both routes) showed an A_2 singlet at δ=4.75 ppm due to the α CH_2 benzylic protons and no singlet at lower field. In contrast dibenzyl-O-trityl-L-malate indicates an A_2 singlet at δ=5.0 ppm for the β CH_2 benzylic protons.

[β-Malyl^1]-angiotensin II. The protected octapeptide was prepared by the solid phase method of Merrifield[8] on a 1% crosslinked chloromethyl polystyrene containing 0.75 meqCl/g (Bio-Beads S-X1; 200–400 mesh). A substitution of 0.67 mmoles of Boc-Phe per g of resin was obtained[9]. The remainder of the synthesis was performed with 1.49 g of substituted resin. The Boc group was used for the protection of the α-amino functions with the side-chain protecting groups indicated in parentheses. Pro, His(Tos), Ile, Tyr(O-2-Br-Z), Val, Arg(NO_2). Couplings were performed with 2.5 equiv. of t-Boc-protected amino acids and DCC (0.25 M in CH_2Cl_2) for 120 min. The completion of the coupling reaction was confirmed by the Kaiser ninhydrin test[10]. For the incorporation of α-benzyl-malate, the corresponding dicyclohexylammonium salt was neutralized, and the oily product was coupled to the heptapeptide polymer using a 3-fold molar excess of DCC and HOBt until a negative ninhydrin test was obtained (5 h) Boc-protecting groups were removed at each stage by treatment with 50% CF_3COOH in CH_2Cl_2 containing 2% anisole (20 ml, 20 min). Neutralization was effected with 10% triethylamine in CH_2Cl_2 (20 ml, 5 min).

Cleavage of the peptide from the resin and removal of the protecting groups was accomplished with HF containing 10% by volume of anisole for 1 h at 0 °C[11]. After removal of HF and drying under vacuum the resin was washed several times with ether and then was extracted with acetic acid (2 M). Lyophilization of the latter extract yielded the crude peptide in a solid form. A portion of this product (~300 mg) was purified by gel filtration on sephadex G-15 column (2.6×100 cm) using 1 M acetic acid as the eluent, followed by partition chromatography on sephadex G-25 M on 2 successive columns using the solvent systems n-butanol-acetic acid-pyridine-water (30:6:20:24) and n-butanol-acetic acid-water (4:1:5). Overall yield was 30% based on the quantity of Boc-Phe originally attached to the

Table 1. Yields and physical properties of compounds **I–VI**

Compd.	Yield, %	Purification process	IR ($CHCl_3$), ν (cm^{-1})	NMR ($cDCl_3$), δ (ppm)
I	70	Column chromatography on silica gel. Eluent: hexane-$CHCl_3$ (7:3). Oil	3540, 1740	2.8 (d, 2 H, $-CH_2CO$), 3.6 (br signal, 1 H, $-OH$, D_2O exchangeable), 4.95–5.00 (two singlets, 4 H, CH_2Ph), 7.2 (s, 10 H, 2 C_6H_5-).
II	50	Gel filtration on Sephadex LH-20. Eluent: ethyl acetate. Oil	2930, 1740, 1450	2.5 (br d, 2 H, $-CH_2CO$), 4.45 (br t, 1 H, $-CHOC$), 4.75 (s, 2 H, α-CH_2Ph), 4,90 (s, 2 H, β-CH_2Ph), 7.2 (br signal, 25 H, 5 C_6H_5-)
III	40 45[a]	Column chromatography on silica gel. Eluent: benzene-ethanol (9:1). Oil	3000–2800, 1740–1720	2.5 (br d, 2 H, $-CH_2CO$), 4.75 (s, 2 H, α-CH_2Ph), 7.2 (br signal 20 H, 4 C_6H_5-)
IV	60 62[a]	Recrystallization from ether. Petroleum ether[b]	2920, 2860, 1760[c]	0.9–2.1 (br signal, 22 H, 2 $C_6H_{11}-$), 2.45 (br d, 2 H, $-CH_2CO$), 4.40 (br t, 1 H, COCHO), 4.70 (s, 2 H, α-CH_2Ph), 7.2 (br signal, 20 H, 4 C_6H_5-)
V	40	Column chromatography on silica gel. Eluent: $CHCl_3$-ethyl acetate (8:2). Oil	3530, 3200-2900, 1740	2.8 (d, J = 6 Hz, 2 H, $-CH_2CO$), 4.45 (t, J = 6 Hz, 1 H, $-COCHO$), 5.05 (s, 2 H, $-CH_2Ph$), 7.2 (s, 5 H, $-C_6H_5$)
VI	35	Recrystallization from ethyl acetate[d]	3300–2800, 1740, 1590	1.0–2.2 (br signal, 22 H, 2 $C_6H_{11}-$), 2.6 (d, J = 6 Hz, 2 H, $-CH_2CO$), 4.4 (t, J = 6 Hz, 1 H, $-COCHO$), 5.1 (s, 2 H, CH_2Ph), 7.25 (s, 5 H, $-C_6H_5$)

[a]Yield by the second route. [b]M.p. 154–155 °C. Analysis calculated for $C_{42}H_{49}O_5N$. Calculated C, 77.89%; H, 7.57%; N, 2.16% Found: C, 78.35%; H, 7.56%; N, 2.05%. [c]IR in KBr. [d]M.p. 140–141 °C. Analysis calculated for $C_{23}H_{35}O_5N$. Calculated: C, 68.14%; H, 8.64%; N, 3.45%. Found: C, 68.32%, H, 8.80% N, 3.53%.

Table 2. Comparative agonist and antagonist effects of analogues of angiotensin II

Angiotensin II analogue	In vivo, rat blood pressure (%)	In vitro, rabbit aorta PD_2^a	PA_2^b	a_E^c	Relative affinity (%)	Binding assay[d] (%)
[Sar^1]-	100	8.21	-	1.0	100	100
[Asn^1]-	16.8	7.25	-	1.0	4.1	31
[β-Mal^1]-	61.5	7.14	-	1.0	2.1	26
[Sar^1, Leu^8]-	NE	-	8.71	0.0	100	
[Asn^1, Leu^8]-	NE	-	7.86	0.0	14	
[β-Mal^1, Leu^8]-	NE	-	7.41	0.0	7.3	3.6

[a]PD_2 is the negative log of the half-maximal dose. [b]The PA_2 value has been defined as the negative log of the molar concentration of a competitive antagonist that reduces the effect of a double concentration of agonist to that of a single one. [c]a_E is the intrinsic activity. [d]Binding assay on adrenal membranes.

resin. TLC: R_f 0.17 (upper phase of the solvent system A; n-BuOH-AcOH-H_2O 4:1:5 v/v); R_f 0.37 (solvent system B; n-BuOH-AcOH-pyridine-H_2O 30:6:20:24 v/v); Mp. 233–235 °C; $[\alpha]_D^{25}$ -60.3° (*c* 0.5, 1 M AcOH). The HPLC profile (Hibar prepacked column RT 250-4, 4 mm × 25 cm Licrosorb RP 18 Merck, load 20–50 µg, flow rate 1.5 ml/min; solvent system: isocratic 17% CH_3CN/0.01 M HCOOH-triethylamine pH 4) revealed that the compound was 99% pure. Amino acid analysis: Arg, 1.04; Val, 1.00; Tyr, 0.97; Ile, 0.97; His, 0.94; Pro, 1.00; Phe, 1.05; high-field magnet fast atom bombardment (FAB-MS) gave $[M+H]^+$ 1047 which is in good agreement with the expected value.

[β-Malyl[1], Leu[8]]-angiotensin II. This analogue was synthesized according to the procedure described above for the [β-Malyl[1]]-AII, starting with 1.75 g (1 mmole) of Boc-Leu-resin. After the purifications, the final product (28% overall yield) gave single spots on TLC: R_f(A) 0.10; R_f(B) 0.39; M.p. 244–246 °C; $[\alpha]_D^{25}$ -86.4° (*c* 0.5, 1M AcOH). The HPLC profile (isocratic 15% CH_3CN/0.01 M HCOOH-triethylamine pH 4) revealed that the compound was 99% pure. Amino acid analysis. Arg, 1.03; Val, 1.02; Tyr, 0.97; Ile, 0.98; His, 0.93; Pro, 1.01; Leu, 1.06; FAB-MS gave $[M+H]^+$ 1013. The instruments used were FINNIGAN-MAT 4600 and KRATOS MS 50- high magnet FAB.

Results and discussion. The biological activities of the synthetic peptides were established with the standard tests in vivo (rat blood pressure) and in vitro on rabbit aorta strips, according to Regoli[12] and shown in table 2. It is seen immediately that [β-Malyl[1]]-AII is a pure agonist and [β-Malyl[1], Leu^8]-AII is a pure inhibitor. Neither of the hydroxy isosters is very potent in comparison to [Sar^1]-agonist and [Sar^1, Leu^8]-antagonist, but they are almost similar to [Asn^1]-analogues. However, both hydroxy isosters are long acting and this may be accounted for by metabolic or enzymatic resistance. In particular, the [β-Mal^1]-AII shows high potency in vivo and very low potency in vitro. The binding data of [β-Mal^1]-AII are definitely much higher compared to [β-Mal^1, Leu^8]-AII (26% against 3.6%). No simple experimental error can account for the unexplained discrepancy so far.

The present data further confirm the results of previous investigations[13] on the importance of the N-terminus α-nitrogen either to bind or form electrostatic hydrogen bonding with polar groups in/or close to the receptor.

1 All optically active amino acids are of the *L* configuration. Abbreviations used follow the recommendations of IUPAC-IUB as found in Biochemistry *14* (1975) 449 and Biochem. J. *126* (1972) 773. Other abbreviations: AII, angiotensin II; HPLC, high pressure liquid chromatography; Mal, malic acid.

2 Acknowledgments. We express our deep appreciation to Dr E. Escher, Department of Pharmacology, University of Sherbrooke, Canada, for the biological assays. Also, we thank Dr J. Yergey and Dr R. Cotter, Department of Pharmacology, the Johns Hopkins University, School of Medicine, USA and Mr P. Warren of Finnigan-Mat San Jose, California, USA, for the determination of M.W. of analogues by FAB-MS.

3 Reprint requests to D.T., Laboratory of Organic Chemistry, Patras University, Patras (Greece).

4 Regoli, D., Rioux, F., Park, W.K., and Choi, C., Can. J. Physiol. Pharmac. *52* (1974) 39.
5 Riniker, B., and Schwyzer, R., Helv. chim. Acta *47* (1964) 2357.
6 Zervas, L., and Theodoropoulos, D., J. Am. chem. Soc. *78* (1956) 1359. Coutsogeorgopoulos, C., and Zervas, L., J. Am. chem. Soc. *83* (1961) 1885.
7 Denham, W.S., and Woodhouse, H., J. chem. Soc. *103* (1913) 1861.
8 Merrifield, R.B., J. Am. chem. Soc. *85* (1969) 2149.
9 Loffet, A., Int. J. Pept. Protein Res. *3* (1971) 297.
10 Kaiser, E., Colescott, R.L., Bossinger, C.P., and Cook, P.I., Analyt. Biochem. *34* (1970) 595.
11 Sakakibara, S., Shimonishi, Y., Kishida, Y., Okada, M., and Sugihara, H., Bull. chem. Soc. Japan *40* (1967) 2164.
12 Regoli, D., Can. J. Physiol. Pharmac. *48* (1970) 481.
13 Regoli, D., Park, W.K., and Rioux, F., Pharmac. Rev. *26* (1974) 69.

0014-4754/83/040353-03$1.50+0.20/0

Identification and efficient synthesis of 6-methoxy-2-benzoxazolinone (MBOA), an insect antifeedant

I. Kubo and T. Kamikawa[1]

Division of Entomology and Parasitology, College of Natural Resources, University of California, Berkeley (California 94720, USA), and Faculty of Science, Osaka City University, Sugimoto-cho, Sumiyoshi-ku, Osaka 558 (Japan), October 13, 1982

Abstract. The identification and an efficient synthesis of 6-methoxy-2-benzoxazolinone (MBOA), an insect antifeedant in *Zea mays* is reported.

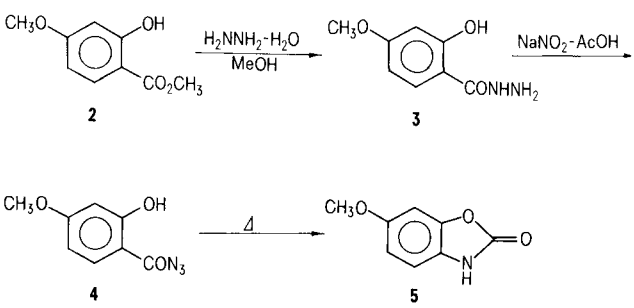
African armyworm, *Spodoptera exempta* larvae feed almost exclusively on graminaceous plants. Nevertheless, during our study some chromatographic fractions of a hexane extract of maize, *Zea mays*, a favored host plant for this insect species, actually deter feeding with leaf disk bioassay[2].

The active antifeedant principle, $C_8H_7O_3N$, m.p. 160–161 °C was identified as 6-methoxy-2-benzoxazolinone (MBOA) by spectroscopic data (UV, IR, MS and NMR). This compound was previously reported as a host plant resistant factor against European corn borer in *Z. mays*[3]. Possibly, this is also the case against African armyworm. ED_{50}-value of MBOA against this pest insect with a feeding assay is 500 ppm. MBOA also exhibits an inhibitory effect on growth of silkworm, *Bombyx mori* larvae with another feeding assay[4]. More recently, MBOA has been suggested to be a naturally occurring environmental cue affecting reproductive cycles in many mammals[5].

This simple chemical structure with unique biological activities is of primary synthetic interest for both determining the structure-activity relationships and elucidating mode of action at the biochemical and physiological levels.

Although the synthesis of MBOA is known[6–8], the reported overall yields are quite low, being 17% at best. In this paper, we report a synthetic scheme with which we were able to obtain 33% yield of MBOA.

In our scheme, commercially available 4-methoxysalicylic acid (**1**) is used as starting material. Brief treatment with diazomethane 1 gave the methyl ester (**2**), which was then converted to the hydrazide (**3**) with hydrazine hydrate in 60% yield. Diazatization of 3 with sodium nitrite gave the crystalline keto azide (**4**) (m.p. 96–97.5 °C) in 72% yield, which on pyrolysis in xylene afforded the isocyanate intermediate by Curtius rearrangement and proceeded with concomitant intramolecular addition of phenolic hydroxyl group to give 6-methoxy-2-benzoxyzolinone (**5**) (m.p. 168–170 °C) in 85% yield. The overall isolated yield of **5** was 33% from **1**. The synthetic products were identical in all respects with the natural product (m.p. and IR- and NMR-spectra).



2 **3** **4** **5**

1 This study was initiated since the hexane extract of *Z. mays* was found to induce biting responses without actual feeding (ingestion) responses. The authors thank A. Chapya and S. Asano for technical assistance.

2 Kubo, I., and Nakanishi, K., Host plant resistance to pests, ACS Symposium Series 62, p. 165. American Chemical Society, Washington, D.C. 1977.
3 Klun, J.A., and Brindley, T.A., J. Econ. Ent. *59* (1966) 711 and references therein.
4 Chan, B.G., Waiss, Jr, A.C., Stanley, W.L., and Goodban, A.E., J. Econ. Ent. *71* (1978) 366.
5 Sanders, E.H., Gardner, P.D., Berger, P.J., and Neugus, N.C., Science *214* (1981) 67 and 69.
6 Smissman, E.E., LaPidus, J.B., and Beck, S.D., J. org. Chem. *22* (1957) 220.
7 Ricky, J.D., Scism, A.J., Caskey, A.L., and BeMiller, J.N., Agric. Biol. Chem. *39* (1975) 683.
8 Allen, E., and Laird, S., J. org. Chem. *36* (1971) 2004.

0014-4754/83/040355-01$1.50+0.20/0