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STRUCTURE AND SYNTHESIS OF ARTHONIN, A LICHEN METABOLITE FROM *ARTHONIA ENDLICHERI*

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Abstract - Arthonin, a metabolite of the lichen *Arthonia endlicheri* has been structurally elucidated as (-)-N-benzoyl-L-valinyl N'-benzoyl-L-isoleucinate. The syntheses of arthonin and iso-arthonin [(-)-N-benzoyl-L-isoleucyl-O-benzoyl-L-valinol] are described.

INTRODUCTION

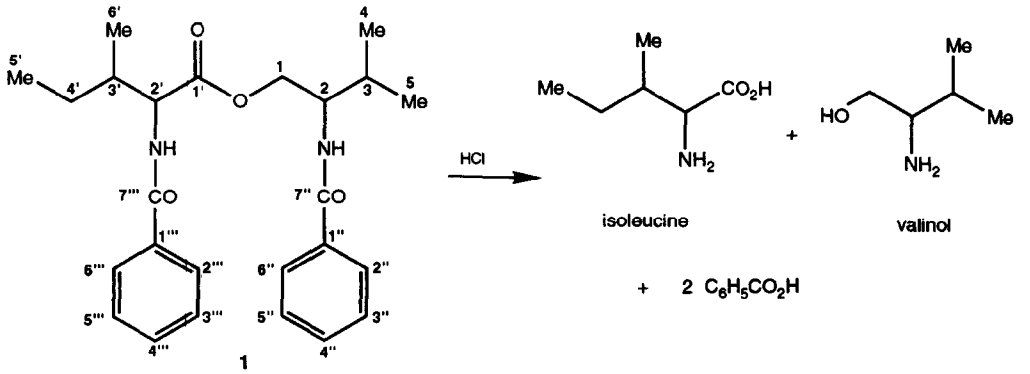
Zopf's collection of lichen substances in the Botanical Museum in Berlin-Dahlem contained a small amount of a compound which he had isolated from *Arthonia endlicheri* (Garov.) Oxner (syn. *A. lobata* (Flot.) Massal.; Arthoniaceae), but did not mention in his monograph[1]. We received this sample through the courtesy of Prof. G. Follmann (at that time in Berlin) and describe here the structure elucidation and synthesis of this lichen metabolite which we name arthonin.

RESULTS AND DISCUSSION

Arthonin, crystallized from methanol-water as thin needles of m.p.165-167 and $[\alpha]_D^{24} \pm 0$ ($CHCl_3$, c 1.09), proved to be a neutral compound and gave a positive Lassaigne test. The IR spectrum (in KBr) showed strong bands, indicative of the presence of benzamide (1530, 1630, 3350 cm^{-1}) and ester (1718 cm^{-1}) groups. The high-resolution mass spectrum gave the formula $C_{25}H_{32}N_2O_4$ (found 424.2366; calculated 424.2362). Hydrolysis of arthonin yielded isoleucine, valinol and benzoic acid, identified by t. l. c. analysis. NMR spectroscopy revealed the complete structure (except the stereochemistry) of arthonin **1**. In addition to aromatic protons the $^1H,^1H$-2D-COSY NMR spectrum of **1** showed two isolated spin systems which could be readily assigned to an isoleucine and a valinol fragment, respectively, based on coupling connectivities. With data from a ^{13}C APT spectrum and a $^1H,^{13}C$ one-bond shift correlation experiment it was possible to assign all carbon signals

In memory to my dear friend Günther Snatzke.

unequivocally. Two proton doublets (δ 6.65 and 6.48) gave no correlation with carbon signals, thus it could be concluded that these signals belong to the NH protons. In the proton detected $^{1}H,^{13}C$ multiple bond correlation spectrum (HMBC) one NH proton (δ 6.65) showed correlations to a benzoyl carbonyl ^{13}C signal as well as to the isoleucine carbonyl ^{13}C signal, whereas the other NH proton (δ 6.48) showed a correlation only with a benzoyl carbonyl ^{13}C signal. These findings confirm the structure **1** of arthonin (Scheme 1).



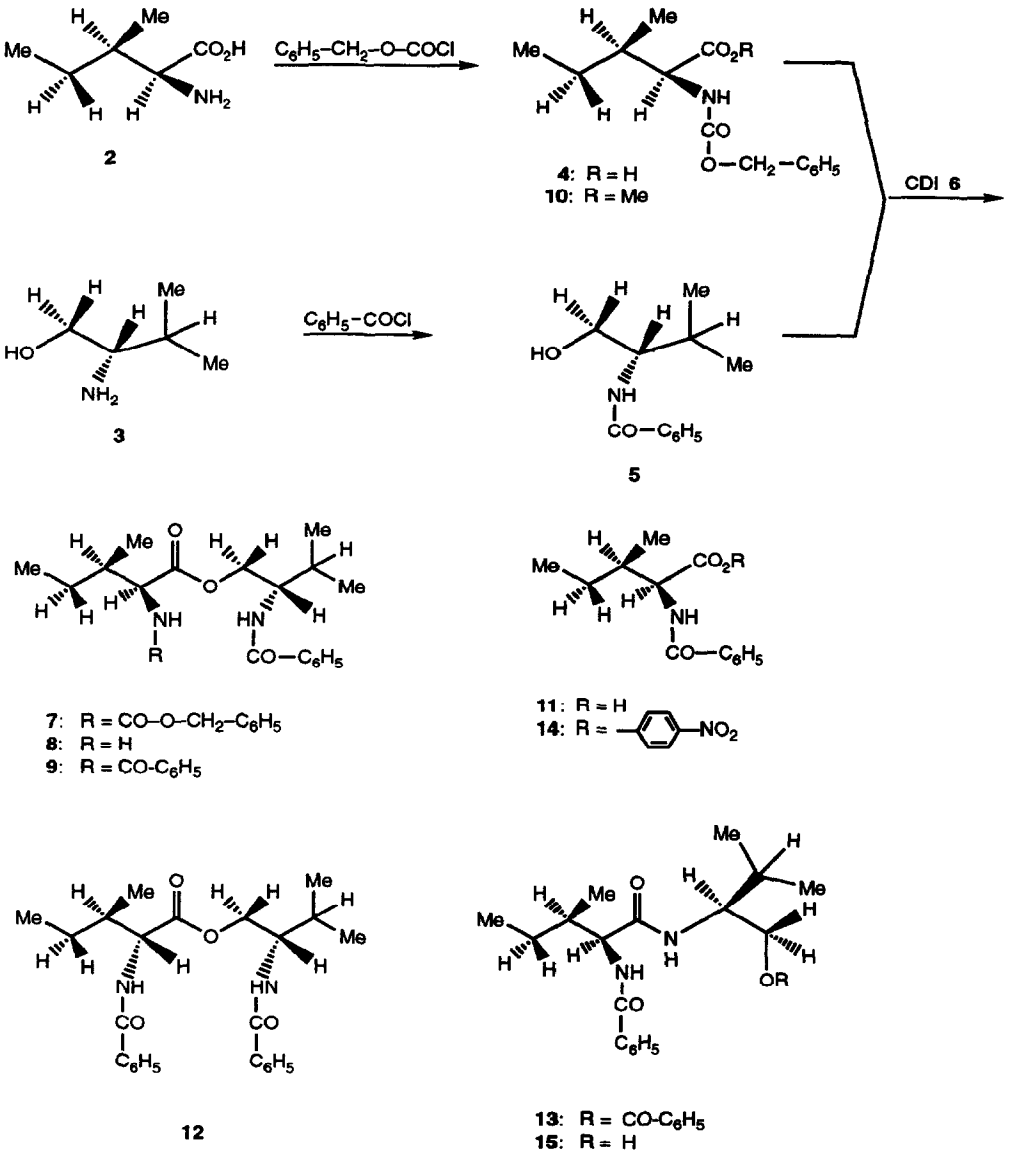
Scheme 1. Structure and hydrolysis of arthonin **1**

With the assumption that L-amino acids were used for biosynthesis in the lichen, we synthesized arthonin from L-isoleucine **2** and L-valinol **3** according to the following scheme (Scheme 2).

(+)-L-Isoleucine **2** was transformed with benzyl chloroformate to the benzyloxycarbonyl derivative **4** and (+)-L-valinol **3** was transformed to (-)-N-benzoyl-L-valinol **5**. Condensation of **4** and **5** in the presence of 1,1'-carbonyldiimidazole (CDI) **6** gave (-)-N-benzoyl-L-valinyl N'-benzyloxycarbonyl-L-isoleucinate **7**. The carbobenzoxy group in **7** was removed with hydrobromic acid in acetic acid to **8** and this amine benzoylated with benzoyl chloride-pyridine to (-)-N-benzoyl-L-valinyl N'-benzoyl-L-isoleucinate **9** which proved to be identical with Zopf's arthonin in all respects except the optical rotation. While Zopf's authentic arthonin showed no optical rotation, the synthetic compound **9** had $[\alpha]_D^{20}$ - 8.8; the only explanation for this discrepancy is that the Zopf compound had been racemised in the course of the time (90 years) since its isolation. Methylation of **4** with diazomethane gave the methyl ester **10**.

Condensation of (+)-N-benzoyl-L-isoleucine **11** with (-)-N-benzoyl-L-valinol **5** in the presence of diimidazole **6** led to a mixture of arthonin and epimer **12**, which could not be separated by crystallization or chromatography. Nevertheless, assignments of all ^{13}C NMR signals of **12** from the APT spectrum of the mixture were possible because the ^{13}C chemical shifts of arthonin were known.

Akaline hydrolysis of **9** gave (+)-N-benzoyl-L-isoleucine **11** and (-)-N-benzoyl-L-valinol **5**.

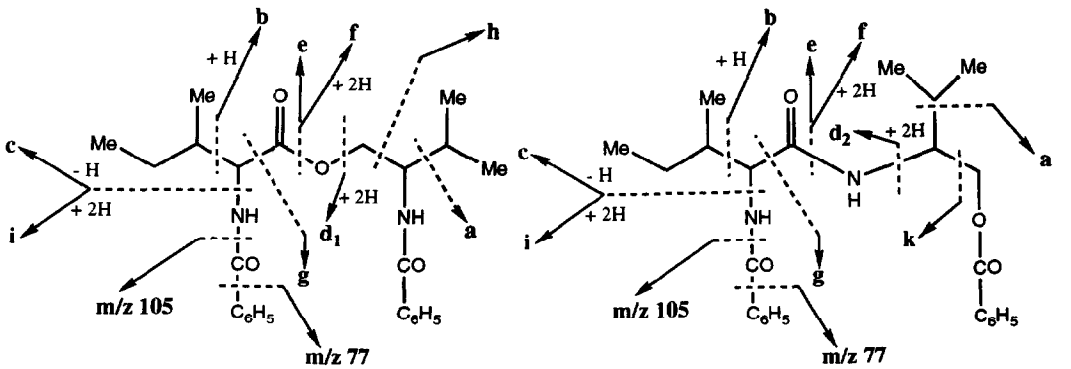


Scheme 2. Synthesis of arthonin **9** and iso-arthonin **13**

To synthesize the isomeric -CO-NH-compound, isoarthonin **13**, (+)-N-benzoyl-L-isoleucine was transformed to the p-nitrophenyl ester **14** and this derivative condensed with **3** by means of N,N'-dicyclohexylcarbodiimide to **15**, which gave with benzoyl chloride in pyridine iso-arthonin.

Arthonin represents the first amino acid and amino alcohol ester derivative to be reported from lichens. Interestingly McCorkindale et al.[2] found a related compound, N-benzoyl-O-[N'-benzoyl-L-phenylalanyl]-phenylalaninol in the fungus *Penicillium canadense*.

The EI mass spectrum of arthonin shows a significant fragmentation locating the several structural subunits (Scheme 3).



arthonin **1** iso-arthonin **13**

Scheme 3. Mass spectral fragmentation of arthonin **1** and iso-arthonin **13**

Fragment ions **a** (m/z 381) and **b** (m/z 368) indicate the presence of an isopropyl and butyl moiety, respectively. The most intense ions, at m/z 77, m/z 105 (base peak) and m/z 122 (**i**) confirmed a benzamide group. While ions of type **g** (m/z 190), **e** (m/z 218) and $\mathbf{d}_1$ (m/z 236) reveal the benzoylated isoleucine moiety, the key ions at m/z 208 (**f**) (complementary to ion **e**) and m/z 176 (**h**) indicate the presence of a benzoylated valinol unit.

In principle the mass spectral fragmentation of iso-arthonin **13** is very similar to that of arthonin **9** as indicated by key ions of type **a**, **b**, **c**, **e**, **f**, **g**, **i**, and m/z 105. However, there are some remarkable differences such as the quite different abundance ratio **a**/**b** and the intense ion at m/z 162. Furthermore, the appearence of key ions **k** (m/z 289) and $\mathbf{d}_2$ (m/z 235, $C_{13}H_{19}N_2O_2$) in **13** is not possible in **9** for structural reasons.

The ^1H and ^{13}C NMR data of arthonin, iso-arthonin and some synthetic percursors are summarized in the Tables 1 and 2, respectively.

Table 1: ^{1}H chemical shifts (ppm), multiplicities and coupling constants (Hz, in parenthesis) of compounds **1**, **5**, **7**, **11**, and **13** #

H	1	5	7 +	11	13
1a	4.493 dd (11.4, 3.1)	3.8 - 3.7 m	overlapped		4.4 - 4.3 m
1b	4.308 dd (11.4, 5.3)	3.8 - 3.7 m	overlapped		4.4 - 4.3 m
2	4.24 m	3.89 m	overlapped		4.20 m
3	2.03 m	2.00 m	1.97 m		1.94 m
4	1.057 d (6.9)*	0.981 d (6.7)*	1.02 d (6.7)		1.026 d (6.7)*
5	1.040 d (6.9)*	0.963 d (6.8)*	1.02 d (6.7)		1.007 d (6.7)*
NH	6.48 br d (ca. 8)	6.77 br d (ca. 9)	6.47 br d (ca. 9)		6.45 br d (ca. 9)
2'	4.680 dd (7.5, 5.8)		overlapped	4.853 dd (8.4, 4.8)	4.600 dd (8.6, 6.0)
3'	1.98 m		1.84 m	2.08 m	2.07 m
4'a	1.53 m		1.36 m	1.58 m	1.54 m
4'b	1.25 m		1.12 m	1.28 m	1.21 m
5'	0.918 t (7.4)		0.83 t (7.1)	0.963 t (7.4)	0.931 t (7.4)
6'	0.978 d (6.8)		0.90 d (6.8)	1.009 d (6.9)	0.932 d (6.8)
NH'	6.65 br d (ca. 8)		5.37 br d (ca. 8.5)	6.86 br d (ca. 8)	6.76 br d (ca. 8)
2"/6"	7.72 d (7.5)	7.73 d (8.3)	7.77 d (7.1)		7.92 d (8.4)
3"/4"/5"	7.5 - 7.3 m	7.5 - 7.3 m	7.5 - 7.3 m		7.5 - 7.2 m
2'"/6'"	7.72 d (7.5)		ca. 7.3 br s	7.80 d (8.5)	7.71 d (8.2)
3'"/4'"/5'"	7.5 - 7.3 m		ca. 7.3 br s	7.5 -7.3 m	7.5 - 7.3 m

Solvent: $CDCl_3$; 200 MHz (**7**, **12**), 300 MHz (**5**, **13**) or 500 MHz (**1**, **11**)

Because it was not possible to separate compound **12** from its epimer **9**, ^{1}H NMR signals of **12** were strongly overlapped with those of **9**. Thus, proton signals of **12** could not be assigned with exception of H-2' (δ 4.831, dd, J = 8.3/4.2 Hz).

+ OCH_2a: δ 5.05 d (J = 12.2Hz); OCH_2b: δ 4.93 d (J = 12.2 Hz)

* Assignments may be reversed in each vertical column

Table 2: ^{13}C chemical shifts (ppm) of compounds **1**, **5**, **7**, **11**, **12** and **13**

C	1	5	7	11	12	13
1	65.3	63.1	65.1		65.3	65.0
2	54.2	57.3	53.9		54.0	53.8
3	29.6	29.0	29.6		29.6	29.6
4	19.5[a]	19.5[a]	19.2[a]		19.4[a]	19.5[a]
5	19.1[a]	19.0[a]	18.9[a]		18.9[a]	18.6[a]
1'	172.2		172.2	175.6	172.0	171.3
2'	57.4		58.5	56.9	56.4	57.4
3'	37.6		37.3	37.8	37.6	37.3
4'	25.4		24.8	25.1	26.3	26.3
5'	11.4		11.2	11.5	11.7	11.6
6'	15.5		15.2	15.3	14.8	14.6
1"	134.4	134.5	134.4		134.4	129.6
2"/6"	127.1	126.9	126.9		127.1	129.6
3"/5"	128.6	128.4	128.3		128.5	128.6
4"	131.8	131.4	131.3		131.8	133.0
7"	167.5[b]	168.3	167.3		167.6[b]	166.6
1"'	133.7		136.0	133.5	133.9	133.8
2"'/6"'	127.0		128.0	127.1	127.0	127.0
3"'/5"'	128.4		128.3	128.5	128.6	128.3
4"'	131.3		127.9	131.8	131.5	131.7
7"'	167.4[b]		c, d	168.0	167.3[b]	167.4

Solvent: $CDCl_3$; 75.5 MHz (**1**, **5**, **7**, **11**, **12**) or 125 MHz (**13**)

[a, b] Assignments may be reversed in each vertical column

c: δ (OCH_2) 66.8; d: δ (CO) 156.1

EXPERIMENTAL

MS: AMD 402 (AMD Intectra GmbH)

Arthonin (authentic material from Zopf's collection) **1**. IR (Unicam SP 200). ν_{max}^{KBr} 704, 730, 760, 790, 810, 850, 940, 990, 1050, 1080, 1150, 1190, 1240, 1306, 1398, 1444, 1458, 1490, 1530, 1572, 1595, 1630, 1658, 1718, 2970, 3350 cm^{-1}. MS m/z (rel. int.) 424.2366 (M^+, 5 %, calc. for $C_{25}H_{32}N_2O_4$ 424.2362), 381.1791 (**a**, 15, calc. for $C_{22}H_{25}N_2O_4$ 381.1814), 368.1759 (**b**, 3, calc. for $C_{21}H_{24}N_2O_4$ 368.1736), 303.1799 (**c**, 14, calc. for $C_{18}H_{25}NO_3$ 303.1834), 249 (2), 236.1271 (**d_1**, 24, calc. for $C_{13}H_{18}NO_3$ 236.1287), 218.1180 (**e**, 40, calc. for $C_{13}H_{16}NO_2$ 218.1181), 208.1314 (**f**, 12, calc. for $C_{12}H_{18}NO_2$ 208.1338), 190.1226 (**g**, 66, calc. for $C_{12}H_{16}NO$ 190.1232), 176.1079 (**h**, 29, calc. for $C_{11}H_{14}NO$ 176.1075), 146.0565 (25, calc. for C_9H_8NO 146.0606), 122.0608 (**i**, 9, calc. for C_7H_8NO 122.0606), 105.0350 (100, calc. for C_7H_5O 105.0340), 77 (22).

(+)-N-Benzyloxycarbonyl-L-isoleucine **4**. (-)-L-Isoleucine (Merck, 99 %, $[\alpha]_D^{20}$ + 36 (c 5 in HCl, 1 mol/l)) (6.55g) was dissolved in 4 N NaOH (12.5 ml) and mixed in several portions at 0 ° in 15 min with benzyl chloroformate (11 g) and 4 N NaOH (12.5 ml) under shaking; shaking was continued at room temperature for additional 20 min. The reaction mixture was extracted with Et_2O (2 x 50 ml), the ether phase separated and the alkaline phase acidified with conc. HCl at 0 ° and extracted with Et_2O (3 x 100 ml). The ethereal extracts were combined, washed with H_2O, dried with Na_2SO_4, the Et_2O removed under vacuum and the residue (15 g) chromatographed over silica gel (50 g, with 5 % H_2O). Elution with n-hexane : Et_2O = 1 : 1 (500 ml) yielded **4** (10 g) as oil of $[\alpha]_D^{20}$ + 16.2 ($CHCl_3$, c 2.2). $C_{14}H_{19}NO_4$ (265.30). MS, m/z 265 (M^+, 53 %), 220 (30, $[M - CO_2H]^+$), 176 $[M - CO_2H - CO_2]^+$, 108 (100, $[C_6H_5\text{-}CH_2OH]^+$), 91 (80, $C_7H_7^+$).

Methylation of **4** with diazomethane in Et_2O gave the methylester **10** as oil. $C_{15}H_{21}NO_4$ (279.33). MS, m/z 279 (M^+, 15 %), 220 (64, $[M - CO_2Me]^+$), 176 (87, $[M - CO_2Me - CO_2]^+$), 108 (57, $[C_6H_5 - CH_2OH]^+$), 91 (100, $C_7H_7^+$).

(-)-N-Benzoyl-L-valinol **5**. From (+)-L-valinol (Fluka, m.p. 30 - 34 °, $[\alpha]_D^{20}$ + 11 (H_2O, c 10) (3.15 g) in pyridine (5 ml) and benzoyl chloride (4.21 g) in several portions at 0 °. After 48 hrs at room temperature and the usual work up, the resulting product was heated under reflux with NaOH (0.6 g) in MeOH : H_2O = 1 : 1 (150 ml) for 1 hr and the MeOH removed under vacuum. The resulting residue was crystallized from benzene and gave **5** in needles of m.p. 100 - 101 ° and $[\alpha]_D^{28}$ - 49.3 ($CHCl_3$, c 1.824). $C_{12}H_{17}NO_2$ (207.27). MS m/z 207 (M^+). IR ν_{max}^{KBr} 430, 530, 660, 785, 830, 870, 890, 920, 975, 1010, 1025, 1070, 1195, 1230, 1280, 1290, 1325, 1345, 1370, 1408, 1440, 1460,1480, 1518, 1525, 1530, 1540, 1575, 1600, 1620, 1685, 2860, 2925, 2950, 2965, 3050,3075, 3280, 3320, 3425, 3525, 3850 cm^{-1}.

(-)-N-Benzoyl-L-valinyl N'-benzyloxycarbonyl-L-isoleucinate **7**. To a solution of (-)-N-benzyloxycarbonyl-L-isoleucine (1.48 g) in ethanol-free $CHCl_3$ (10 ml) 1,1'-carbonyldiimidazole (0.9 g) was added in several portions; after 1 hr at room temperature (-)-N-benzoyl-L-valinol (1.16 g) was added in several portions and the mixture kept at room temperature for 5 days. After this time, the $CHCl_3$ was removed under vacuum, the residue dissolved in Et_2O and washed with 10 % $NaHCO_3$ in H_2O (20 ml). The Et_2O phase was dried with Na_2SO_4, the solvent removed under vacuum and the residue chromatographed over silica gel (50 g, with 5 % H_2O). n-Hexane : Et_2O = 4 : 1 (800 ml) eluted **7** as needles of m.p. 110 - 111 ° and $[\alpha]_D^{20}$ - 27.7 ($CHCl_3$, c 0.55). $C_{26}H_{34}N_2O_5$ (454.55). MS, m/z 454 (M^+, 10 %), 411 (8), 347 (4), 289 (5), 254 (5), 208 (7), 189 (36), 176 (63), 146 (83), 105 (100).

(-)-N-Benzoyl-L-valinyl N'-benzoyl-L-isoleucinate **9**. A solution of (-)-N-benzoyl-L-valinyl N'-benzyloxycarbonyl-L-isoleucinate (0.75 g) in a solution of HBr in acetic acid (33 %, 30 ml) was kept at room tempera-

ture for 2 hrs, the solvent removed under vacuum, the residue dissolved in pyridine (15 ml), mixed with benzoyl chloride (2 ml) at 0 ° and the solution kept at room temperature for 24 hrs. After the usual work up the resulting residue was chromatographed over 20 g silica gel (with 5 % H_2O). n-Hexane : Et_2O = 25 : 1 (500 ml) eluted an oil, n-hexane : Et_2O = 23 : 2 (500 ml) a crystalline compound (not further investigated) and $CHCl_3$ (500 ml) compound **9**, from MeOH needles of m.p. 167 -168 ° and $[\alpha]_D^{20}$ -8.8 ($CHCl_3$, c 1.18). $C_{25}H_{32}N_2O_4$ (424.53). MS, m/z 424 (M^+, 7 %), 381 (26), 368 (4), 303 (18), 249 (2), 236 (17), 218 (32), 208 (10), 190 (49), 176 (21), 146 (11), 122 (5), 105 (100), 77 (24). IR (Zeiss Specord) ν_{max}^{KBr} 452, 530, 602, 640, 665, 680, 720, 740, 790, 860, 920, 940, 960, 975, 990, 1020, 1070, 1140, 1180, 1230, 1290, 1302, 1330, 1352, 1385, 1465, 1480, 1515, 1525, 1530, 1575, 1600, 1625, 1635, 1725, 2975, 2925, 2970, 3350 cm^{-1}.

[α]	-9.3	-10.0	-16.1	-16.9	-21.2.
	578	546	436	406	366 nm

Alkaline hydrolysis of synthetic arthonin **9**. Synthetic arthonin (0.085 g) was heated under reflux with NaOH (0.062 g) in a mixture of H_2O (20 ml) and MeOH (15 ml) for 16 hrs, the solvent removed under vacuum, the residue extracted with Et_2O (3 x 20 ml, A), the alkaline phase acidified with 10 % H_2SO_4 and again extracted with Et_2O (3 x 20 ml, B). Extract A gave after crystallization from benzene hair-like needles of m.p. 98 - 99 ° and $[\alpha]_D^{22}$ -20.9 ($CHCl_3$, c 0.715), identical with (-)-L-benzoylvalinol. The acid fraction B of $[\alpha]_D^{21}$ + 30.9 ($CHCl_3$, c 2.31) was identical with (+)-N-benzoyl-L-isoleucine.

(+)-N-Benzoyl-L-isoleucine **11**. From (+)-L-isoleucine (3.93 g), benzoyl chloride (3.45 ml) and 10 % NaOH in the usual way; after chromatography m.p. 90-92 ° and $[\alpha]_D^{28}$ + 31.6 ($CHCl_3$, c 2.25).

[α]	33.7	41.3	69.7	86.6	121.7.
	578	546	436	406	366 nm

IR, ν_{max}^{KBr} 525, 550, 612, 650, 665, 705, 800, 910, 925, 975, 1000, 1025, 1070, 1125, 1150, 1175, 1215, 1270, 1290, 1325, 1380, 1412, 1450, 1480, 1518, 1525, 1575, 1600, 1640, 1690, 1710, 1720, 2875, 2930, 2960, 3310, 3850 cm^{-1}.

(-)-N-Benzoyl-L-isoleucyl-O-L-valinol **15**. (+)-N-Benzoyl-L-isoleucine (0.474 g) and p-nitrophenol (0.2874 g, m.p. 102 - 107 °) were dissolved in ethyl acetate (20 ml) and mixed with N,N'-dicyclohexylcarbodiimide (0.420 g) at 0 ° and the mixture kept at this temperature for 30 min. The precipitate of dicyclohexylurea was removed by filtration and the filtrate mixed with (-)-L-valinol (0.245 g). After 24 hrs at room temperature the mixture was concentrated to a volume of 3 ml, the precipitate removed by filtration and crystallized from benzene-$CHCl_3$: **15** in hair-like needles of m.p. 170 - 171 ° and $[\alpha]_D^{23}$ -31.3 ($CHCl_3$, c 0.79); yield:

0.0757 g. $C_{18}H_{28}N_2O_3$ (320.42). MS, m/z 321 (0.67 $[M + H]^+$), 320 (0.31, M^+), 290 (15), 264 (3), 234 (4), 224 (7), 218 (34), 190 (100), 169 (8), 162 (17), 143 (5), 105 (67), 77 (16). IR ν_{max}^{KBr} 570, 630, 660, 780, 865, 880, 915, 970, 1015, 1060, 1150, 1220, 1240, 1305, 1340, 1375, 1460, 1518, 1525, 1530, 1540, 1575, 1625, 2845, 2870, 2925, 2960, 3275 (-NH), 3450 (-OH), 3850 cm-1 (OH).

(-)-N-Benzoyl-L-isoleucyl-O-benzoyl-L-valinol, iso-arthonin **13**. To a solution of **15** (0.05 g) in pyridine (4 ml), benzoyl chloride (0.5 ml) was added and the mixture kept at room temperature for 24 hrs. The usual work up and crystallization from Et_2O gave iso-arthonin in crystals of m.p. 204 - 206 ° and $[\alpha]_D^{22}$ - 24.2 ($CHCl_3$, c 0.43). MS m/z 424.2354 (M^+, 4 %, calc. for $C_{25}H_{32}N_2O_4$ 424.2362), 381 (**a**, 1), 368 (**b**, 17), 303 (**c**, 2), 289.1869 (**k**, 3, calc. for $C_{17}H_{25}N_2O_2$ 289.1916), 246.1387 (**b**-$C_6H_5CO_2H$, 2, calc. for $C_{14}H_{18}N_2O_2$ 246.1368), 235.1430 ($\mathbf{d}_2$, 5, calc. for $C_{13}H_{19}N_2O_2$ 235.1447), 218.1194 (**e**, 17, calc. for $C_{13}H_{16}NO_2$ 218.1181), 208 (**f**, 3), 190 (**g**, 90), 176 (2), 162.0933 (**g** - C_2H_4, 15, calc. for $C_{10}H_{12}NO$ 162.0919), 134 (2), 122 (3), 105 (100), 77 (24), 72 (10).

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