Tetrahedron. Vol. 31, pp. 2629 to 2632. Pergamon Press 1975. Printed in Great Britain

THE ABSOLUTE CONFIGURATION OF RHIZOBITOXINE

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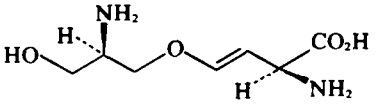
(*Received in USA* 17 *January* 1975; *Received in UK for publication* 1 *April* 1975)

Abstract—The absolute configuration of rhizobifoxine, an amino acid produced by *Rhizobium japonicum*, has been determined by means of a new chiroptical method.

Rhizobitoxine is an amino acid which is produced by *Rhizobium japonicum*.[1] This antimetabolite[2] was found to cause the symptoms of rhizobial-induced chlororis in *Glycine max.* L. Merrill (soybean).[1] It also irreversibly inactivates β-cystathionase in bacteria[3] and plants[4] and inhibits the conversion of methionine into ethylene in plants.[5] Recently, rhizobitoxine was identified as 2-amino-4-(2-amino-3-hydroxypropoxy)-*trans*-but-3-enoic acid.[6] However, the stereochemistry of the two chiral centers in the molecule remained to be established. We have now determined the absolute configuration of rhizobitoxine as shown in structure **1**.

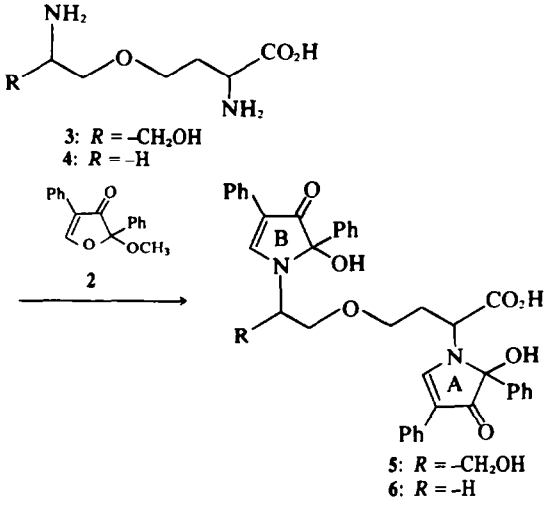


1

In the preceding paper,[7] we have outlined a new method for determining the absolute configuration of α-amino acids. Thus, α-amino acids react with 2-methoxy-2,4-diphenyl-3(2*H*)-furanone (**2**, MDPF) to form *N*-substituted 3,5 - diphenyl - 5 - hydroxy - 2 - pyrrolin - 4-ones. The sign of the extremum at 390 nm (first Cotton effect) in the CD spectra of such adducts is solely dependent on the absolute configuration at the α-carbon of the parent amino acid.[7] Pyrrolinones derived from L-amino acids exhibit a positive first Cotton effect, while those derived from D-amino acids show a negative first Cotton effect.

Dihydrorhizobitoxine (**3**),[8] a natural congener of rhizobitoxine and also of unknown stereochemistry, lends itself readily to derivatization with MDPF (**2**). Thus, treatment of **3** with MDPF (**2**) gives the dipyrrolinone adduct **5** with two chiroptically active groups, A and B. The CD spectrum of **5** should be the sum of additive increments from both of these chromophores.† With appropriate models it should be possible to predict the respective contributions of A and B to the first Cotton effect of **5** and thus to choose the correct stereostructure of **3** from the four possible isomers. Since rhizobitoxine (**1**) is readily converted to dihydrorhizobitoxine (**3**) by hydrogenation of the enol ether linkage,[6] the method should yield its stereostructure as well.

The dipyrrolinone adduct **6**, synthesized from L-2-amino-4-(2-aminoethoxy)butanoic acid (**4**)[9] and MDPF (**2**), has a CD-maximum at 390 nm with a molar ellipticity (θ_{390}) of +6300. Thus, it was predicted that chromophore A would contribute +6300 to θ_{390} in the CD spectrum of **5** if **3** is an L-amino acid, and −6300 if **3** is a D-amino acid.



3: $R = -CH_2OH$
4: $R = -H$

2

5: $R = -CH_2OH$
6: $R = -H$

In order to determine the sign of the Cotton effect for typical amino alcohol adducts, the pyrrolinones **10**, **11** and **15** were synthesized as shown in Scheme 1. The CD spectra of both *S*-adducts, **11** and **15**, exhibit minima at 385 nm while the *R*-adduct **10** has a maximum at 386 nm. From the data obtained with **15**, it was predicted that pyrrolinone B would contribute +4500 to θ_{390} in the CD-spectrum of **5** if the amino alcohol moiety in **3** has the *R*-configuration and −4500 if it has the *S*-configuration.

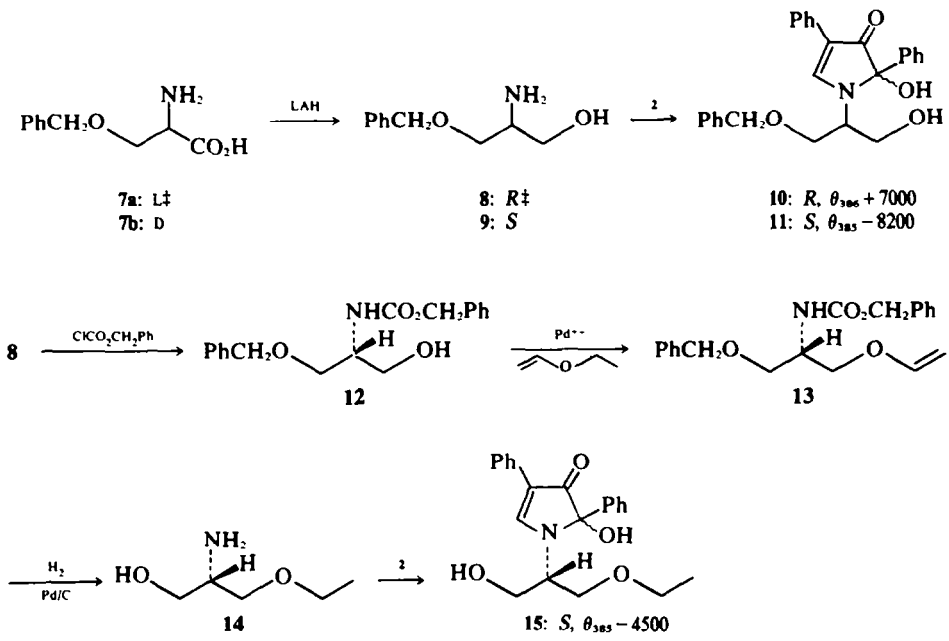
The predicted sign and magnitude of the molar ellipticity for the four possible structures **16**–**19** of dihydrorhizobitoxine are compiled in Table 1. The molar ellipticity (θ) of the dipyrrolinone **5**, derived from naturally occurring dihydrorhizobitoxine, was found to be +12,000 (393 nm). Thus, dihydrorhizobitoxine has structure **17**.

Reduction of rhizobitoxine with hydrogen over palladium gave rise to an amino acid which appeared to be identical to dihydrorhizobitoxine by thin layer chromatography. The reduced amino acid was condensed with MDPF (**2**) yielding an adduct **5** which was identical to that derived from dihydrorhizobitoxine by IR and NMR spectroscopy. The value of θ for this substance was found to be +12,800 (390 nm). Thus, the reduced amino acid also has structure **17**, and rhizobitoxine has structure **1**.

EXPERIMENTAL

M.ps are uncorrected and were determined on a Kofler Hot Stage apparatus. IR spectra were recorded on either a Perkin–Elmer 621 or a Beckman IR-9 spectrophotometer. NMR spectra were recorded on a Varian T-60 and HA-100 instruments and are reported in ppm, from internal TMS. CD spectra were recorded on a Durrum–Jasco Spectropolarimeter, Model ORD/CD/UV-5. Elemental analyses were carried out under the supervision of Dr. F. Scheidl (of our Microanalytical laboratory).

†This also assumes that the two chromophores, A and B, are far enough apart to be independent of each other.



Scheme 1.

‡The conventional symbols L and D will be used to denote the stereochemistry of α-amino acids, while the method of Cahn, Ingold and Prelog[10] (*R* and *S*) will be used to denote the stereochemistry of amino alcohols.

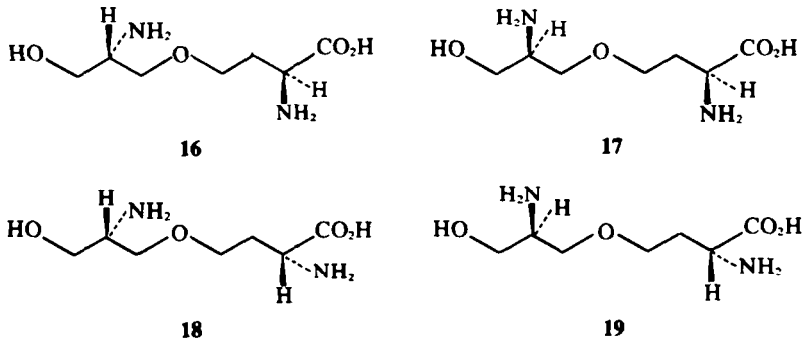


Table 1.

Structure	Configuration and Contribution to θ_{390} Chromophore A	Chromophore B	Predicted Molar Ellipticity (θ_{390}) of 5
16	L, +6300	*S*, −4500	+1800
17	L, +6300	*R*, +4500	+10800
18	D, −6300	*S*, −4500	−10800
19	D, −6300	*R*, +4500	−1800

2S,7 - *Bis*(2,3 - *dihydro* - 2,4 - *diphenyl* - 2 - *hydroxy* - 3 - *oxo* - 1 - *pyrrolyl*) - 5 - *oxoheptanoic acid* (6)

To a vigorously stirred soln at 55° consisting of 4 (0·19 g; 1 mmol), 20 ml water, 10 ml MeOH and Et_3N (0·2 ml; 2.14 mmol) was added 2 (1·6 g; 6 mmol) in 35 ml hot MeOH. After addition was completed, stirring was continued for another 30 min at 55°. The mixture was then allowed to cool to room temp. The suspended solid was removed by filtration and the filtrate was concentrated *in vacuo*. The concentrate was taken up in 100 ml water containing 0·3 ml Et_3N. The resulting aqueous soln was washed twice with 200 ml ether. The organic extracts were washed with two 35 ml portions water, each containing 0·1 ml Et_3N. The aqueous layers were combined, acidified with 8 ml 1 *N* HCl and extracted twice with 200 ml EtOAc. The organic extracts were washed with water (100 ml), dried over Na_2SO_4, and evaporated *in vacuo*.

The residue was chromatographed on silica gel (130 g) using $CHCl_3$/MeOH as the eluent. The fractions eluted with 75:25 $CHCl_3$/MeOH were concentrated *in vacuo*. The resultant solid was repeatedly triturated with ether yielding 0·4 g of 6 (63%): IR (KBr) 1670, 1605, 1577 cm^{-1}; UV (EtOH) λ_{max} 217 nm (ε 23, 500), 280 (32,250), 384 (12,800); CD (0·01 *M*, EtOH) $[\theta]_{445}$ 0, $[\theta]_{378}$ +6300 (max), $[\theta]_{288}$ +18,000 (max), $[\theta]_{262}$ 0, $[\theta]_{250}$ −1500 (min), $[\theta]_{230}$ 0, $[\theta]_{215}$ +18,000 (max).

R-2-*Amino*-3-*benzyloxypropanol* (8)

To a stirred suspension of LAH (2·92 g; 76·0 mmol) in 70 ml dry THF under argon at room temp. was added small portions of finely divided *O*-benzyl-L-serine[11] (5·0 g; 25·6 mmol). After the addition was complete, stirring was continued at room temp. for 1 hr and then at reflux temp. for 2 hr. The mixture was cooled to 0°, diluted

with 140 ml ether, and the excess LAH destroyed with wet ether followed by water. The solids were removed by filtration and the resultant soln concentrated *in vacuo* yielding an oil. The oil was dissolved in ether and the soln was treated with charcoal, filtered through Celite, dried over Na_2SO_4, and concentrated *in vacuo* yielding 4·4 g of **8** (95%) as an oil. The alcohol **8** was characterized as the hydrochloride salt: m.p. 135–137°; $[\alpha]_D^{25}$ −8·40° (H_2O, c = 1·059); IR (KBr) 3375, 3050 (broad), 1600, 1520, 1480, 1460, 1380, 1260, 1030, 1020, 940, 740 cm^{-1}; NMR ($CDCl_3$) δ 7·86 (s, 5H, $PhCH_2$–), 5·00 (s, 2H, $PhCH_2$–), 4·12 (m, 5H, $-CH_2CHCH_2$–). (Found: C, 54·89; H, 7·57; N, 6·32; Cl, 16·32. Calc. for $C_{10}H_{15}NO_2$·HCl: C, 55·17; H, 7·41; N, 6·43; Cl, 16·29%).

S-2-*Amino*-3-*benzyloxypropanol* (**9**)

Alcohol **9** was made from *O*-benzyl-D-serine[11] (20 g, 102·4 mmol) using the method described for the synthesis of the *R* isomer **8**. A yield of 17·4 g (93·2%) was obtained. The material was characterized as the hydrochloride salt: m.p. 135–137°; $[\alpha]_D^{25}$ +7·76° (H_2O, c = 0·967). (Found: C, 54·94; H, 7·45; N, 6·34; Cl, 16·50. Calc. for $C_{10}H_{15}N_2O_2$·HCl: C, 55·17; H, 7·41; N, 6·43; Cl, 16·29%).

2R-(2,3-*Dihydro* - 2,4 - *diphenyl* - 2 - *hydroxy* - 3 - *oxo* - 1 - *pyrrolyl*) - 3 - *benzyloxypropan* - 1 - *ol* (**10**)

To a soln consisting of **8**·HCl (0·436 g; 2 mmol), 25 ml MeOH, and Et_3N (0·3 ml; 2·14 mmol) was added **2** (0·6 g; 2·25 mmol). After the mixture had been stirring at room temp. for 12 hr, the solvent was removed *in vacuo*. The residue was chromatographed on 150 g silica gel using $CHCl_3$/MeOH (97·5:2·5; v/v) as eluent. The fractions containing **10** were concentrated *in vacuo* and the residue was taken up in ether. The ether soln was treated with activated charcoal, filtered, and concentrated *in vacuo* yielding 0·72 g of **10** (87%): IR (KBr) 1665, 1607, 1570 cm^{-1}; UV (EtOH) λ_{max} 279 nm (ϵ 17,160), 382 (6220); CD (0·01*M*, EtOH) $[\theta]_{450}$ 0, $[\theta]_{386}$ +7000 (max), $[\theta]_{341}$ 0, $[\theta]_{323}$ −2400 (min), $[\theta]_{308}$ −1800 (max), $[\theta]_{263}$ −7800 (min), $[\theta]_{245}$ 0, $[\theta]_{216}$ +11,500 (max).

2S - (2,3 - *Dihydro* - 2,4 - *diphenyl* - 2 - *hydroxy* - 3 - *oxo* - 1 - *pyrrolyl*) - 3 - *benzyloxypropan* - 1 - *ol* (**11**)

Pyrrolinone adduct **11** was made from **9**·HCl (0·436 g; 2 mmol) using the method described for the synthesis of the *R* isomer **10**. The product (0·655 g, 79%) was obtained as an amorphous powder (**11**): IR (KBr) 1665, 1608, 1570 cm^{-1}; UV (EtOH) λ_{max} 279 nm (ϵ 18,580), 382 (6900); CD (0·01*M*, EtOH) $[\theta]_{422}$ 0, $[\theta]_{385}$ −8200 (min), $[\theta]_{341}$ 0, $[\theta]_{323}$ +2700 (max), $[\theta]_{294}$ +1800 (min), $[\theta]_{263}$ +7700 (max), $[\theta]_{245}$ 0, $[\theta]_{220}$ −11,500 (min).

R - 1 - *Benzyloxy* - 3 - *hydroxy* - 2 - *propylcarbamic acid benzyl ester* (**12**)

To a soln of **8** (35·7 g; 0·195 mol) and Na_2CO_3 (41·5 g; 0·494 mol) in 60 ml water was slowly added benzyl chloroformate (39·0 g; 0·217 mol). The mixture was stirred for 22 hr, saturated with NaCl and extracted with several portions of EtOAc. The EtOAc extracts were combined and dried over Na_2SO_4. The solvent was removed *in vacuo* yielding an oil which solidified. The solid was crystallized from ether/light petroleum yielding 60·4 g of **12** (97%): m.p. 38–41°; $[\alpha]_D^{25}$ +11·40° ($CHCl_3$, c = 1·01); IR ($CHCl_3$) 3640, 3500, 3450, 1720, 1520, 1460, 1345, 1315, 1245, 1085, 1030 cm^{-1}; NMR ($CDCl_3$) δ 7·34 (s, 5H, $PhCH_2$–), 7·32 (s, 5H, $PhCH_2$–), 5·50 (broad, 1H, NH), 5·10 (s, 2H, $PhCH_2$–), 4·51 (s, $PhCH_2$–), 3·80 (m, 5H, $-CH_2CHCH_2$–), 2·66 (broad, 1H, OH). (Calc. for $C_{18}H_{21}NO_4$: C, 68·55; H, 6·71; N, 4·44. Found: C, 68·69; H, 6·81; N, 4·46).

S-1-*Benzyloxy*-3-*vinyloxy*-2-*propylcarbamic acid benzyl ester* (**13**)

Alcohol **12** (5·35 g, 17 mmol) was dissolved in 40 ml dry glyme and 60 ml dry ethyl vinyl ether. Under N_2, the soln was cooled to −20° with stirring and a mixture of NaH_2PO_4 (9·3; 77·5 mmol) and $Pd(Cl)_2(PhCN)_2$[12] (6·48 g; 16·9 mmol) was added. The mixture was stirred at −20° for 4·5 hr, and diluted with 5 ml pyridine and 100 ml ether. The solids were removed by filtration, and the filtrate concentrated *in vacuo*. The residue was dissolved in ether and the soln treated with charcoal. The charcoal was removed by filtration through Celite and the filtrate concentrated *in vacuo* yielding **13** (5·41 g; 93%). An analytical sample of **13** was prepared by crystallization from light petroleum: m.p. 27–29°; $[\alpha]_D^{25}$ −2·13° ($CHCl_3$, c = 1·034); IR ($CHCl_3$) 3445, 3025, 2950, 2875, 1720, 1620, 1520, 1455, 1320, 1210, 1060 cm^{-1}; NMR ($CDCl_3$) δ 7·35 (s, 5H, $PhCH_2$–), 7·32 (s, 5H, $PhCH_2$–), 6·44 (dd, 1H, J = 7 and 14 Hz, $-CH{=}CH_2$), 5·25 (broad, 1H, NH), 5·08 (s, 2H, $PhCH_2$–), 4·50 (s, 2H, $PhCH_2$–), 4·4–3·5 (m, 7H, $-CH{=}CH_2$ and $-CH_2CHCH_2$–). (Found: C, 70·34; H, 6·81; N, 4·37. Calc. for $C_{20}H_{23}NO_4$: C, 70·36; H, 6·79; N, 4·10).

S-2-*Amino*-3-*ethoxypropanol* (**14**)

Ether **13** (1·81 g, 5·3 mmol) was dissolved in 20 ml EtOH under N_2 and 10% Pd-C (0·25 g) was added. The mixture was stirred under H_2 for 1·5 hr. At this time 2 ml conc. HCl was added to the mixture and the stirring was continued an additioal hr under H_2. The mixture was filtered through Celite and concentrated *in vacuo*. The residue was applied to an ion exchange column (AG® 50W-X4; 100–200 mesh; H^+ form) and the amino alcohol eluted with 1·5*N* NH_4OH. The solvent was removed *in vacuo* yielding a crystalline residue. Sublimation under vacuum at room temp. gave 0·302 g of **14** (48%): m.p. 43–47°; $[\alpha]_D^{25}$ −1·88° ($CHCl_3$, c = 0·799); IR ($CHCl_3$) 3625, 3475, 3375, 3000, 2975, 2930, 2875, 1680, 1120, 1010 cm^{-1}; NMR ($CDCl_3$) δ 3·50 (*m*, 6H, $-CH_2CHCH_2$– and CH_3CH_2–), 3·07 (q, 1H, $-CH_2CHCH_2$–), 2·17 (broad, 3H, OH and NH_2), 1·19 (t, 3H, CH_3CH_2–). (Found: C, 50·24; H, 10·88; N, 11·68. Calc. for $C_5H_{13}NO_2$: C, 50·40; H, 11·00; N, 11·75%).

2S - (2,3 - *Dihydro* - 2,4 - *diphenyl* - 2 - *hydroxy* - 3 - *oxo* - 1 - *pyrrolyl*) - 3 - *ethoxypropan* - 1 - *ol* (**15**)

To a soln consisting of **14** (0·20 g; 1·7 mmol) and 20 ml methanol was added **2** (0·532 g; 2 mmol). After stirring at room temp. for 6 hr, the solvent was removed *in vacuo* and the residue processed as described for **10**. The product (0·459 g, 81%) was obtained as an amorphous powder (**15**): IR (KBr) 1670, 1608, 1575 cm^{-1}; UV (EtOH) λ_{max} 279 (ϵ 16,700), 382 (6020); CD (0·01*M*, EtOH) $[\theta]_{450}$ 0, $[\theta]_{385}$ −4500 (min), $[\theta]_{342}$ 0, $[\theta]_{325}$ +1700 (max), $[\theta]_{293}$ 0, $[\theta]_{283}$ −1200 (min), $[\theta]_{278}$ 0, $[\theta]_{263}$ +2300 (max), $[\theta]_{246}$ 0, $[\theta]_{223}$ −3200 (min).

Reduction of rhizobitoxine

Rhizobitoxine (7 mg, 0·037 mmol) was dissolved in 1 ml of EtOH/water (1:1, v/v) and 4 mg 10% Pd/C was added. The mixture was stirred under H_2 for 2 hr, filtered through Celite, and concentrated *in vacuo* yielding a gum (6·5 mg, 0·034 mmol, 92%) which was nearly pure by TLC [silica gel; ethanol (32), water (8), NH_4OH (1)]. This material was used for derivatization with MDPF (**2**) without further purification.

2S, 7R - *Bis*(2,3 - *dihydro* - 2,4 - *diphenyl* - 2 - *hydroxy* - 3 - *oxo* - 1 - *pyrrolyl*) - 8 - *hydroxy* - 5 - *oxaoctanoic acid* (**5**)

(A) *From natural dihydrohizobitoxine.*[8] To a soln at 55° consisting of dihydrorhizobitoxine (7 mg), water (1·1 ml), MeOH (0·5 ml), and Et_3N (0·05 ml) **2** (60 mg) was added **2** (60 mg) in 1·5 ml warm MeOH. Stirring was continued for 15 min. Another portion of **2** (30 mg) was then added and the resultant soln was stirred for an additional 15 min. The mixture was then taken up in 25 ml water containing 1·5 ml 1*N* HCl. The aqueous soln was washed with two 30 ml portions EtOAc. The combined organic extracts were washed with two 20 ml portions water, dried (Na_2SO_4), and concentrated *in vacuo*. The residue was chromatographed on 15 g silica gel. Elution with $CHCl_3$/MeOH (75:25; v/v) gave the bis-adduct **5**. Concentration of these fractions gave a solid which was triturated with ether yielding 27 mg of **5**: IR (KBr) 1663, 1605, 1570, 1495, 1455, 1370, 1235, 1175, 1105, 1070, 1028, 960, 910, 790, 755, 699 cm^{-1}; UV (EtOH) λ_{max} 281 nm (ϵ 29,360), 385 nm (11,000); CD (0·001*M*, EtOH) $[\theta]_{440}$ 0, $[\theta]_{394}$ +12,000 (max), $[\theta]_{349}$ 0, $[\theta]_{325}$ −6800 (min), $[\theta]_{304}$ 0, $[\theta]_{292}$ +6800 (max), $[\theta]_{270}$ 0, $[\theta]_{264}$ −1600 (min), $[\theta]_{239}$ 0, $[\theta]_{214}$ +6000 (max).

(B) *From semi-synthetic dihydrorhizobitoxine.* Dihydrorhizobitoxine, obtained by reducing rhizobitoxine, was derivatized with MDPF (**2**) in the same manner as the natural dihydrorhizobitoxine (see above, Part A). The product had the following spectral properties: IR (KBr) 1668, 1608, 1570, 1498, 1455, 1388, 1235, 1180, 1110, 1070, 1030, 960, 915, 790, 760, 700

cm^{-1}; UV (EtOH) λ_{max} 281 nm (ϵ 31,700), 384 (11,980); CD (0·001*M*, EtOH) $[\theta]_{440}$ 0, $[\theta]_{390}$ +12,800 (max), $[\theta]_{346}$ 0, $[\theta]_{322}$ −7800 (min), $[\theta]_{302}$ 0, $[\theta]_{288}$ +10,000 (max), $[\theta]_{260}$ +3000 (min), $[\theta]_{221}$ +18,000 (max).

Acknowledgements—We are indebted to Dr. L. D. Owens of the Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland, for samples of dihydrorhizobitoxine and rhizobitoxine. We thank Dr. V. Toome for chirospectral measurements.

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