ALKALOIDS OF *Buxus sempervirens* var *rotundifolia* BAILLON*

Le thi Thien HUONG, Zdeno VOTICKÝ and Vladimír PAULÍK

Institute of Chemistry,
Slovak Academy of Sciences, 809 33 Bratislava

Received July 3rd, 1980

Twenty-one alkaloids were isolated from the leaves of *Buxus sempervirens* var. *rotundifolia* BAILLON. Buxaminol-B, cyclobuxine-D, cyclovirobuxine-D, cycloprotobuxine-C, buxamine-E, buxtauine-M and buxpiine-K were those already known. New alkaloids, the constitution and configuration of which were inferred from physical data and corroborated by interconversion and correlation, were denominated buxithienine-M and cyclobuxophylline-O. The remaining bases were characterized by physicochemical constants and/or spectral data.

Constituents of leaves and bark of boxwood (*Buxus sempervirens*) were for a long time used in curing various diseases[1]. Recently, it has been shown that buxus alkaloids reveal a strong inhibition of the enzyme cholinesterase[2], and pure cyclobuxine-D, the principal alkaloid of boxwood has been used as a complexing agent to study the reversible helix–coil transition of deoxyribonucleic acid[3]. This paper deals with bases isolated from *B. sempervirens* var. *rotundifolia* BAILLON.

Dry ground leaves of this plant were macerated by a procedure described in[4] and the obtained mixture of alkaloids dissolved in chloroform was extracted with dilute hydrochloric acid. Alkaloids of this part were distributed according to their basicity with McIlvain buffer solutions into separate portions (pH 6·5–3 and 2% hydrochloric acid), from which they were separated by a procedure given in our preceding papers[4].

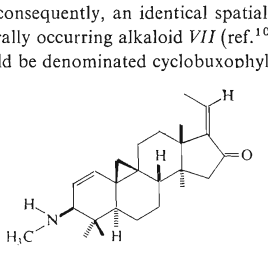
The chloroform solution, from which dilute hydrochloric acid did not extract further alkaloids any more, contains further new bases and was evaporated. The solution of the residue in dilute hydrochloric acid was precipitated with Mayer's reagent[5] to an iodomercuri complex from which the alkaloids were liberated with an ion exchanger and separated by chromatographic techniques. The least polar alkaloid of molecular weight 367·2865 (M^+, for $C_{25}H_{37}NO$ calculated 367·2875) showed in its mass spectrum peaks of ions at m/z M−15, M−28, M−29, M−43, 44, 57, 70; the last three of them characterized the presence of a methylamino group at $C_{(3)}$ (ref.[6]). Its ultraviolet spectrum displayed absorption bands typical of a carbonyl

* Part XIX in the series Alkaloids from *Buxus sempervirens*; Part XVIII: Chem. Zvesti *34*, 706 (1980).

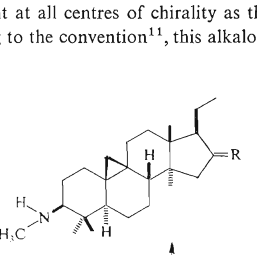
group in conjugation with double bond (λ_{max} 241·5 nm, log ε 4·62) and an isolated double bond (λ_{max} 203 nm, log ε 3·94), the infrared spectrum revealed vibration bands diagnostic of a carbonyl group at a 5-membered ring (1 718 cm^{-1}), a $C_{(1)}$—$C_{(2)}$ double bond[7] (1 642 cm^{-1}) a methylene in a cyclopropane ring (ν_{as} (C—H) 3016, δ(C—H) 1 442 cm^{-1}) and a secondary amine (3 420 cm^{-1}). The ^{1}H-NMR spectrum showed signals of protons of a methylene group in cyclopropane ring (ppm, δ: 0·28 and 0·60, 2 H, ABq, J = 5 Hz), of four tertiary C-methyl groups (0·78, 3 H, s; 0·96, 6 H, d, J = 2·5 Hz; 1·31, 3 H, s). The multiplicity of the signal at 0·96 with a low coupling constant evidenced the presence of a geminal dimethyl group, which was localized from biochemical consideration at $C_{(4)}$. The shift of the A part of the AB doublet into higher field indicated the proximity of a double bond. The signal at 1·80 (3 H, d, J = 8 Hz) was ascribed to methyl protons of the ethylidene grouping, that at 2·45 (3 H, s) to a methylamino group at $C_{(3)}$, those at 5·74 and 5·63 (2 H, q, ABX system, J_{AB} = 8 Hz) to olefinic protons, that at 3·20 (1 H, m) to a proton in the neighbourhood of nitrogen (1 H, m, X part), that at 6·53 (1 H, q, J = 7·5 Hz) to a vinyl proton of the *s-cis* ethylidene grouping at the cyclopentanone ring of the steroid backbone[8]. Basing upon these arguments one is entitled to ascribe the structural formula *I* to this alkaloid. The index of unsaturation indicates that three double bonds are involved in this molecule. Tetrahydro derivative *II* showed in its mass spectrum peaks of ions at m/z 371 (M^+), M−15, M−28, M−29, M−43, 44, 57 and 70. Its ultraviolet spectrum did not contain an absorption band associated with the conjugated double bond of the starting material, whilst a new band ascribable to an isolated carbonyl group (λ_{max} 204 nm, log ε 3·62) appeared. The infrared spectrum revealed the presence of a carbonyl group on a five-membered ring (1 714 cm^{-1}) and a methylene in a cyclopropane grouping (1 439 cm^{-1}); in contrast to the original substance, *II* did not absorb at 1 642 cm^{-1} ($C_{(1)}$—$C_{(2)}$ double bond). The hexahydro derivative *III*, characterized by mass spectrum, disclosed peaks of ions at m/z 373 (M^+), M−15, M−43, 44, 57 and 70. Catalytic hydrogenation of this type of alkaloids proceeds stereoselectively to form 17β-ethyl derivative of 5α-androstane[9]. The correctness of the suggested structure of alkaloid *I*, which was denominated buxithienine-M, was proved by interconversion of *I* and cyclovirobuxine-D (*IV*): chromic oxidation of *IV* followed by a $C_{(20)}$-deamination afforded, similarly as with cyclobuxine-D (ref.[9]), a mixture of *Z*- and *E*-cyclobuxophyllines-M (*Va,b*), which was, without being separated, catalytically stereoselectively hydrogenated to *II* identical with tetrahydrobuxithienine-M. Buxithienine-M is another alkaloid of the α,β-unsaturated cyclopentanone group, which might be artifact[8].

The second alkaloid isolated from this portion had molecular weight 355·2869 (M^+, for $C_{24}H_{37}NO$ calculated 355·2875); further peaks in the mass spectrum were at m/z M−15, M−28, M−29, M−43, and 43. The series of peaks at m/z 30, 43, 56 indicated a primary amino group at $C_{(3)}$. Its ultraviolet spectrum showed an absorption band of a carbonyl conjugated with a double bond (λ_{max} 242 nm, log ε 4·14).

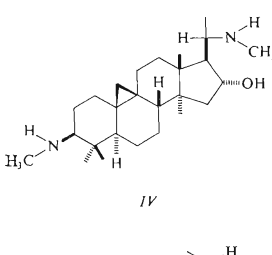
Vibration bands in the infrared region localized the carbonyl group at the five-membered ring (1726 and 1718 cm^{-1}) and also evidenced the presence of a $C_{(17)}$—$C_{(20)}$ double bond (1641 cm^{-1}), a methylene in a cyclopropane ring (1460 cm^{-1}), and a primary amino group (3412 cm^{-1}). The ^{1}H-NMR spectrum displayed signals of methylene protons of a cyclopropane ring at 0·33 and 0·60 (2 H, ABq, J = 5 Hz), of four tertiary C-methyl groups (0·81, 0·93, 1·0, 1·3; 4 × 3 H, ss), of a methyl group protons associated with an *s-cis* ethylidene grouping at 6·51 (1 H, J = 7 Hz) and of protons of an amino group at 8·25 (2 H, broad singlet). These data were close to those of alkaloid *I*, and are in agreement with the proposed structure *VI*. The dimethyl derivative *VII*, prepared by N-methylation of *VI* had the same constants and consequently, an identical spatial arrangement at all centres of chirality as the naturally occurring alkaloid *VII* (ref.[10]). According to the convention[11], this alkaloid should be denominated cyclobuxophylline-O.



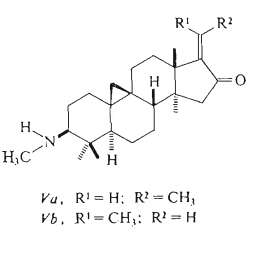
I



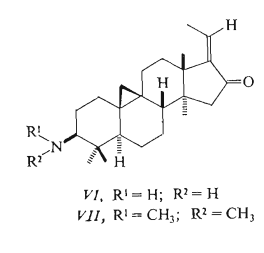
II, R = O
III, R = H, OH



IV



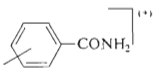
Va, R^1 = H; R^2 = CH_3
Vb, R^1 = CH_3; R^2 = H



VI, R^1 = H; R^2 = H
VII, R^1 = CH_3; R^2 = CH_3

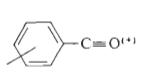
Further six alkaloids of this portion, B/1, B/4 – B/8, were characterized by physicochemical constants and spectral data. Alkaloids isolated from the separate pH portions of the part extractable with dilute hydrochloric acid were identified by spectral means, melting point, and by optical rotation (Table I).

Alkaloid A/3 displayed in its mass spectrum a peak of molecular ion at m/z 598·39810 (for $C_{35}H_{54}N_2O_6$ calculated 598·39814) and further peaks at m/z M – CH_3, M – OH, M – OCH_3, 511 ($C_{31}H_{45}NO_5$), 496 (511 – CH_3), 493 (511 – H_2O), 478 (496 – H_2O). The base peak was formed by the species m/z 72 ($C_4H_{10}N$), characteristic of a dimethylamino group at $C_{(20)}$. Peaks of ions at m/z 198 ($C_9H_{12}NO_4$, *a*) 181 ($C_9H_9O_4$, *b*) and 153 ($C_8H_9O_3$, *c*) were diagnostic of trihydroxybenzoic acid dimethyl ether. Due to the fact that no peaks of an amino function at $C_{(3)}$ were present in the spectrum, this acid can be attached to this position as an amide. The infrared spectrum contained vibration bands of an amido group at 1670 cm^{-1} (ν(C=O), amide I), 1500 cm^{-1} (δ(N—H), amide II), and 1221 cm^{-1} (ν(C—N), amide III). Other bands indicated the presence of a secondary hydroxyl group (1040 and 3508 cm^{-1}), a secondary amino group (3420 cm^{-1}) and a methylene in a cyclopropane ring (1460 cm^{-1}). The base A/5 has embodied, like A/3, dimethyl ether of trihydroxybenzoic acid, since its mass spectrum also contained peaks of fragment ions *a* and *b*.



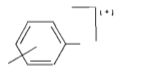
2 CH_3O—, HO—

a



2 CH_3O—, HO—

b



2 CH_3O—, HO—

c

EXPERIMENTAL

Melting points were determined on a Kofler micro hot-stage, optical rotations were measured with a Perkin–Elmer polarimeter, model 141, in 1-cm cells. The separate spectra were recorded with following apparatuses: AEI-MS, model 902 (70 eV, 100 μA, ⟨150–290°C⟩, electron impact mass spectra), Perkin–Elmer, model 457 (KBr, infrared spectra), Beckman DB-GT (methanol, ultraviolet spectra), Tesla BS, model 487 B (80 MHz, $CDCl_3$, tetramethylsilane, ^{1}H-NMR spectra). Aluminium oxide (Reanal, neutral) was used as a carrier for both column and thin-layer chromatography without binder in solvent systems: chloroform–benzene–ethanol 14 : 6 : 5 (S_1), 13 : 10 : 2 (S_2), 14 : 8 : 3 (S_3), 15 : 9 : 1 (S_4), 10 : 8 : 2 (S_5), 14 : 9 : 2 (S_6), chloroform–benzene–methanol 14 : 6 : 5 (S_7). Chromatograms were sprayed with Dragendorf reagent.

Isolation of Alkaloids

Terminal twigs of *Buxus sempervirens* var. *rotundifolia* BAILLON were collected in the Arboretum, Slovak Academy of Sciences, Mlyňany, at the end of October 1975. The dried ground leaves (3·1 kg) were macerated with 50% aqueous methanol acidified with acetic acid, polysaccharides

were removed from the concentrated extract according to the procedure reported earlier[4] and the ammonia alkalized solution was extracted with chloroform; the first crop of alkaloids was extracted from the chloroform solution with dilute hydrochloric acid (A), the second part, which remained in chloroform was obtained as a residue after removing the solvent under diminished pressure (B).

The first part (A, 40·3 g) was worked up in a routine way[4] into portions soluble in McIlvain buffer solutions of pH 6·5, 6·0, 5·0, 4·0, 3·0 and in 2% hydrochloric acid; the separate alkaloids were obtained by column chromatography. The second part (B, 32 g) was stepwise dissolved in 2% hydrochloric acid (30 × 20 ml), from which the alkaloids were precipitated with Mayer's reagent in form of an iodomercuri complex (37 g). This was filtered off, washed with water, dissolved in acetone–methanol–water 6 : 2 : 1 (60 ml) and treated with Amberlite IRA-402 (200 ml, $Cl^{(-)}$) for 48 h with stirring. Alkaloids obtained after filtration of ion exchanger, alkalization and evaporation of the solvent were separated by column chromatography. Separation and quantitative distribution of alkaloids are surveyed in Tables I and II.

TABLE I

Separation of Alkaloids Present in Portion A

Extract pH (g)	Al_2O_3 g, activity (fractions, ml)	Eluent	Combined fractions	Alkaloid	Amount mg
6·5	165, III	*a*	67—71	buxaminol-B	22
(5·5)	(50)			A/1	3·1
	(100)	*b*	85—91	cyclobuxine-D	70·6
			92—101	cyclovirobuxine-D	36
				cyclobuxine-D + cyclovirobuxine-D	131
6·0	1 500, VI	*c* + *b*	15—36	cycloprotobuxine-C	108
(3·5)	(120)		45—49	buxamine-E	121
	(200)	*e*	76	A/2	2·8
5·0	250, VI	*c* + 1%[a]	3—4	buxamine-E	205
(5·0)	(100)		5—6	buxtauine-M	239
		c + 2%[a]	29—35	A/3	59·7
4·0	172, VI	*c* + 1%[a]	3	cyclobullatine-A	22·5
(2·9)	(100)		4	A/4	45
			5	buxpiine-K	94
			18—19	A/5	4·2
3·0	60, VI	*c* + 1%[a]	8—17	cyclovirobuxine-C	5·9
(1·05)	20)				
2% HCl	4·5, VI	*c* + 1%[a]	5	cyclobuxine-D + cyclovirobuxine-D	10·3
(0·7)	(20)				

[a] Diethyl ether, [b] chloroform, [c] benzene, [d] methanol, [e] benzene–chloroform–diethyl ether 1 : 2 : 3.

Characterization of Isolated Alkaloids

Buxithienine-M (I): m.p. 166—169°C (acetone + 5% methanol), $[\alpha]_D^{22}$ —75·6° (*c* 1·2, chloroform), R_F 0·34 (S_4), 0·52 (S_2).

Hydrogenation of buxithienine-M: Adams catalyst (50 mg) was added to the solution of *I* (101 mg) in acetic acid (20 ml) and the mixture was hydrogenated at room temperature for 28 h. The catalyst was filtered off, the filtrate diluted with water (190 ml), basified with ammonia and extracted with chloroform. Yield 102 mg. Thin-layer chromatography of the product showed the presence of two compounds; separation on alumina (activity grade VI, 50 g, eluens dichloromethane) afforded tetrahydrobuxithienine-M (*II*) (45·5 mg), m.p. 144—147°C (dichloromethane), $[\alpha]_D^{22}$ —37·8° (*c* 0·96, chloroform), R_F 0·45 (S_4), *m/z* 371 (M^+) and hexahydrobuxithienine-M (*III*) (9·8 mg), m.p. 280°C (decomp.) (dichloromethane), $[\alpha]_D^{22}$ +16·1° (*c* 0·18, chloroform), R_F 0·35 (S_4), *m/z* 373 (M^+).

Transformation of cyclovirobuxine-D (IV) *to the ketone* II: these operations were analogous to those of cyclobuxine-D (ref.[9]). Yield 27 mg (45%) of ketone *II* (from 65 mg of *IV*) identical (m.p., mixed m.p., optical rotation, R_F) with tetrahydrobuxithienine-M.

Cyclobuxophylline-O (V): m.p. 219—222°C (acetone), $[\alpha]_D^{22}$ —61·5° (*c* 1·1, chloroform), R_F 0·34 (S_4), 0·44 (S_2).

N,N-*Dimethylcyclobuxophylline*-O (cyclobuxophylline-K, *VI*): solution of *V* (22 mg) in formic acid (85%, 1 ml) and formaldehyde (36%, 1 ml) was heated on a steam bath for 2 h, diluted with water (5 ml), alkalized with ammonia and extracted with chloroform. Yield 22 mg (93%), m.p. 193—196°C (acetone), $[\alpha]_D^{22}$ —67° (*c* 0·8, chloroform), R_F 0·88 (S_4), *m/z* 383 (M^+). Cyclobuxophylline-K (ref.[10]): m.p. 194—196°C, $[\alpha]_D$ —72° (*c* 0·52, chloroform).

Alkaloid B/1: m.p. 280°C (decomp.), (acetone + 5% methanol), $[\alpha]_D^{22}$ +11·8° (*c* 0·55, methanol), R_F 0·37 (S_6), *m/z* 353 (M^+), M—15, M—28, M—29, M—43, 44, 57, 70; the last three peaks and the lowered intensity of the last two peaks indicated the presence of a methylamino group

TABLE II

Separation of Alkaloids Present in Portion B

Eluent[a]	Combined fractions	Alkaloid	Amount mg
Benzene	8—34	buxithienine-M	504
	35—56	B/6	7
Benzene–methanol	67—74	buxithienine-M	482
	75—80	B/7	2·9
Methanol	87—90	cyclobuxophylline-O	22
	91—97	B/4	7·5
		B/5	9·2
	98—101	B/1	8·8

[a] Carrier alumina (920 g, activity grade III, fractions 25 ml).

at $C_{(3)}$ adjacent to a $C_{(4)}$-exocyclic double bond[6]. UV spectrum: λ_{max} 243 nm (log ε 4·19, a carbonyl group conjugated with double bond).

Alkaloid B/4: m.p. 150—152°C (chloroform), $[\alpha]_D^{22}$ +16° (*c* 0·4, chloroform), R_F 0·45 (S_2), *m/z* 399 (M^+), M—15, M—28, M—43, M—45, M—59, 297, 72, 58.

Alkaloid B/5: m.p. 179—182°C (chloroform), $[\alpha]_D^{22}$ +9·6° (*c* 0·5, chloroform), R_F 0·33 (S_2), *m/z* 387 (M^+), M—15, M—31, M—33, 72, 58.

Alkaloid B/6: m.p. 214—216°C (ethanol), $[\alpha]_D^{22}$ +6·6° (*c* 0·15, methanol), R_F 0·58 (S_2), *m/z* 426 (M^+), 388, 381, 84, 71, 58. IR spectrum, cm^{-1}: 3518 (ν(O—H)), 1052 (ν(C—O), 2969 (ν_{as} CH_3), 1688 ($\nu(C_{(6)}=C_{(7)})$), 1452 (ν_{as} CH_2 (cyclopropane)).

Alkaloid B/7: m.p. 245—247°C (acetone), R_F 0·32 (S_2), *m/z* 369 (M^+), M—15, M—43, M—29, M—28, 70, 57.

Alkaloid B/8: m.p. 265°C (acetone + 5% methanol), $[\alpha]_D^{22}$ +34·6° (*c* 0·54, chloroform), R_F 0·32 (S_2), *m/z* 384 (M^+), M—15, M—30, M—58 (methylamino group at $C_{(20)}$, 58, 71, 84 (dimethyl group at $C_{(3)}$).

Buxaminol-B: m.p. 194—198°C (methanol), $[\alpha]_D^{22}$ +34·0° (*c* 1·5, chloroform), R_F 0·83 (S_7), *m/z* 414 (M^+); ref.[12]: m.p. 199—200°C (methanol–water), $[\alpha]_D$ +38° (chloroform).

Cyclobuxine-D: m.p. 239—242°C (ethanol), $[\alpha]_D^{22}$ +90·7° (*c* 1, chloroform), R_F 0·52 (S_1), *m/z* 386 (M^+); ref.[13]: m.p. 244—245°C (decomp.) (methanol), $[\alpha]_D^{23}$ +98° (*c* 4·4, chloroform).

Cyclovirobuxine-D: m.p. 220—221°C (acetone + 1% methanol), $[\alpha]_D^{22}$ +60° (*c* 1, chloroform), R_F 0·37 (S_1), *m/z* 402 (M^+); ref.[14]: m.p. 221—224°C (decomp.), $[\alpha]_D$ +63° (chloroform).

Cycloprotobuxine-C: m.p. 199—202°C (acetone), $[\alpha]_D^{22}$ +73°, (*c* 1, chloroform), R_F 0·49 (S_2), *m/z* 400 (M^+); ref.[15]: m.p. 200—202°C, $[\alpha]_D$ +76° (*c* 1, chloroform).

Buxamine-E: amorphous, $[\alpha]_D^{22}$ +35° (*c* 0·4, chloroform), R_F 0·45 (S_6), *m/z* 384 (M^+); ref.[12]: amorphous, $[\alpha]_D^{20}$ +32° (*c* 0·6, chloroform), *m/z* 384 (M^+).

Buxtauine-M, *cyclobullatine*-A, *buxpiine*-K: constants characterizing these alkaloids were identical with those published in our previous papers[16—18].

Alkaloid A/1: m.p. 195—197°C (dichloromethane), R_F 0·17 (S_7), *m/z* 368 (M^+), M—15, M—29, 58, 71, 84 (dimethylamino group at $C_{(3)}$, ref.[6]).

Alkaloid A/2: m.p. 138—142°C (acetone + 5% methanol), R_F 0·79 (S_7), *m/z* 429 (M^+).

Alkaloid A/3: m.p. 272—274°C (acetone + 10% methanol), $[\alpha]_D^{22}$ +20° (*c* 0·25, ethanol), R_F 0·46 (S_6), *m/z* 598 (M^+).

Alkaloid A/4: m.p. 252—256°C (dichloromethane), $[\alpha]_D^{22}$ —109°C (*c* 0·51, methanol), R_F 0·42 (S_3), *m/z* 428 (M^+), M—43, 58, 71, 84.

Alkaloid A/5: m.p. 125—127°C (dichloromethane), $[\alpha]_D^{22}$ —10° (*c* 0·3, dichloromethane), R_F 0·5 (S_3), *m/z* 680·4406 (M^+, for $C_{40}H_{60}N_2O_7$ calculated: 680·4405).

Our thanks are due to Dr L. Dolejš, Institute of Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague for measuring mass spectra.

REFERENCES

1. Schlittler E., Heusler K., Friedrich W.: Helv. Chim. Acta *32*, 2209 (1949).
2. Vincent D., Parant M.: C.R. Soc. Biol. *148*, 1878 (1954).
3. Mahler H. R., Dutton G.: J. Mol. Biol. *10*, 157 (1964).

4. Bauerová O., Votický Z.: Pharmazie *28*, 212 (1973).
5. Hanč O.: *Chemická laboratorní příručka*, p. 511. Průmyslové vydavatelství, Prague 1951.
6. Dolejš L., Hanuš V. Votický Z., Tomko J.: This Journal *30*, 2869 (1965).
7. Jones N. R., Herling F.: J. Org. Chem. *19*, 1252 (1954).
8. Votický Z., Paulík V.: Chem. Zvesti *26*, 376 (1972).
9. Nakano T., Votický Z.: J. Chem. Soc. *1970*, 590.
10. Nakano T., Terao S., Saeki Y.: J. Chem. Soc. C, *1966*, 1412.
11. Tomko J., Votický Z. in the book: *The Alkaloids*, Vol. XIV (R. H. F. Manske, Ed.), p. 5. Academic Press, New York 1973.
12. Stauffacher D.: Helv. Chim. Acta *47*, 968 (1964).
13. Brown K. S. jr, Kupchan S. M.: J. Amer. Chem. Soc. *86*, 4414 (1964)
14. Brown K. S. jr, Kupchan S. M.: Tetrahedron Lett. *1964*, 2895.
15. Nakano T., Hasegava M.: Tetrahedron Lett. *1964*, 3679.
16. Votický Z., Tomko J., Dolejš L., Hanuš V.: This Journal *30*, 3705 (1965).
17. Votický Z., Bauerová O., Paulík V.: This Journal *40*, 3055 (1975).
18. Votický Z., Tomko J.: Tetrahedron Lett. *1965*, 3579.

Translated by Z. Votický.