Tetrahedron Vol. 48, No. 17, pp. 3577-3584, 1992
Printed in Great Britain

0040-4020/92 $5.00+.00
Pergamon Press Ltd

NOVEL TRITERPENOIDS FROM THE ROOTS OF *BUXUS PAPILLOSA*

Atta-ur-Rahman*, Habib Nasir, Erfan Asif, S. Safdar Ali and Zahida Iqbal
H. E. J. Research Institute of Chemistry, University of Karachi
Karachi -75270, Pakistan

M. Iqbal Choudhary[1] and Jon Clardy*
Baker Laboratory, Department of Chemistry, Cornell University
Ithaca, NY 14853-1301, U. S. A.

(*Received in UK* 4 *March* 1992)

Abstract: Three new compounds have been isolated from the roots of *Buxus papillosa*. (+)-Buxapentalactone (**1**) and (+)-buxaheptalactone (**2**) are the rearranged triterpenoid lactones while (+)-3-deoxybuxandonine (**3**) is a 4,4-bisnortriterpenoidal alkaloid. The structures of these steroidal compounds were determined by using X-ray diffraction and spectroscopic techniques.

Introduction

Buxus papillosa C. K. Schneider (Buxaceae) has been used for the treatment of malaria, rheumatism and skin diseases.[2] We have reported over 50 new steroidal alkaloids from its leaves. [2-4] Work on the roots of this plant has resulted in the isolation of the non-nitrogenous triterpeniod (-)-buxatenone (**4**). [5] In this paper, we report the isolation and structure elucidation of the rearranged triterpenoid lactones (+)-buxapentalactone (**1**) and (+)-buxaheptalactone (**2**), and the 4,4-bisnortriterpenoidal alkaloid (+)-3-deoxybuxandonine (**3**). Their structures were elucidated on the basis of spectral data and single crystal X-ray diffraction analysis.

Results and Discussion

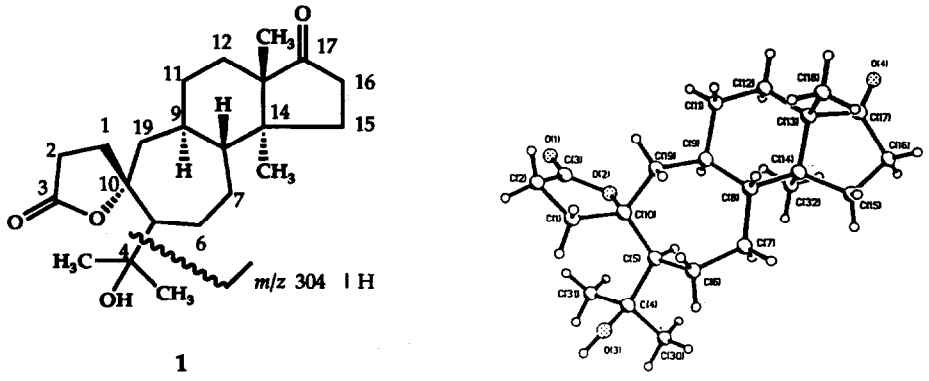
B. papillosa roots were collected from Northern Pakistan. An ethanolic extract was evaporated and partitioned between chloroform and aqueous acid solution at various pH values. These fractions were subjected to column and thin-layer chromatography to afford **1**, **2** and **3**.

The first compound, (+)-buxapentalactone (**1**), was obtained as colorless crystals. The HREIMS spectrum of **1** revealed the M^+ peak at m/z 362.2465 having the molecular composition $C_{22}H_{34}O_4$. The IR spectrum (KBr) revealed the presence of two carbonyl groups at 1730 (five membered ketone[5]) and 1742 (five membered lactone[6]) cm^{-1}. Other absorptions were at 3350 (OH), 2900 (CH) and 1090 (CO) cm^{-1}.

Structure **1** for (+)-3-buxpentalactone was conclusively established by single crystal X-ray diffraction. A suitable crystal was mounted in a capillary due to the hygroscopic nature of the crystalline sample. Compound **1** crystallized in the orthorhombic space group $P2_12_12_1$ with a = 9.505(1) Å, b = 14.185(2) Å, c = 14.6772(2) Å, Z= 4. All unique reflections were collected (2θ<112°) using θ:2θ scans with graphite monochromated Cu-Kα radiation (1.54178 Å). Of the 1497 unique reflections, 1209 (81%) were judged observed with $|F_o| > 5\sigma(F_o)$. The structure was solved by direct methods and refined by using full-matrix least-squares techniques to final discrepancy index of 4.38 % (R_w= 6.15%) for the observed data. Anisotropic heavy atoms and isotropic riding hydrogens were used in refinements.[7] A computer generated perspective drawing of final X-ray model is given in Figure **1**.[8] The minimum energy conformation of **1** was determined with the MM2 program by using crystal coordinates, and shown in Figure **2**.[9]

The ^{1}H-NMR spectrum ($CDCl_3$, 400 MHz) of **1** showed four quaternary methyl signals at δ 0.83, 0.99, 1.27 and 1.29. Four 1H multiplets of an isolated AA'BB' spin system (δ 1.62, 1.74, 2.19 and 2.39) were assigned to the C-15 and C-16 methylene protons. The clear appearance of another AMBB' spin system indicated the presence of a $-CH_2-CH_2-C=O$ group. A multiplet at δ 2.65 was assigned to the C-1 methylene protons, while the C-2 protons appeared as multiplets at δ 1.75 and 3.02. Strong COSY interactions in the COSY-45° spectrum further proved the above mentioned assignments. The ^{13}C-NMR spectrum ($CDCl_3$, 100 MHz) of **1** showed 22 carbon resonances. The carbon multiplicity was determined by DEPT and broad-band decoupled ^{13}C-NMR spectra and showed the presence of four methyl, nine methylene, three methine and six quaternary carbons. The signals at δ 219.0 and 177.0 were assigned to ketone and lactone carbonyl carbons respectively. The resonance at δ 91.9 was assigned to the C-10 spiro quaternary carbon, and the unusually far downfield methylene signal was assigned to C-19 due to its disposition in the anisotropic deshielding region of lactone oxygen diamagnetic cone. The peaks at δ 177.0, 28.8, 29.7 and 91.9 were characteristic of a δ-lactone functionality. The proton-carbon connectivities were established through a hetero-COSY NMR experiment.[10,11] Table **1** summarizes the ^{13}C- and ^{1}H-NMR assignments for **1**.

The mass spectrum (HREIMS) of the (+)-buxapentalactone (**1**) showed the molecular ion at m/z 362.2465, corresponding to a molecular formula $C_{22}H_{34}O_4$ (calcd. 362.2457). The fragment ion at m/z 347.2215 ($C_{21}H_{31}O_4$) was due to the loss of a methyl group from M^+, while m/z 344 represented the loss of H_2O from M^+. The base peak at m/z 304.2062 resulted from the loss of the C-5 side chain. Structure **1** was assigned for (+)-buxapentalactone on the basis of the above studies. The absolute configuration as shown was assumed.



1

Figure 1: A structural drawing of (+)-buxapentalactone is shown on the left and a perspective drawing of the final X-ray model is given on the right. The absolute configuration shown is assumed.

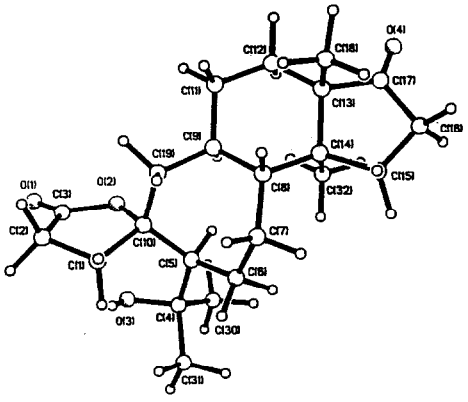
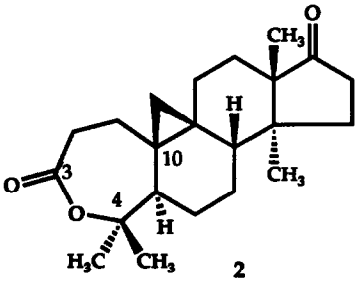


Figure 2 : Minimum energy conformation of 1 (38.97 Kcal) determined with the MM2 program

The second compound isolated from the roots of *B. papillosa* was assigned as (+)-buxaheptalactone (2). HREIMS indicated a molecular formula of $C_{22}H_{32}O_3$ and UV spectrum showed only end absorption. The IR spectrum (KBr) displayed two distinct carbonyl absorptions at 1725 and 1710 cm^{-1}, indicative of a ketone and a lactone carbonyl respectively. Absorption at 1103 cm^{-1} was assigned to a C-O stretching. High resolution mass spectrometry indicated that (+)-buxaheptalactone (2) had the molecular formula $C_{22}H_{32}O_3$ (m/z 344.2356, calcd. 344.2351). The base peak at m/z 96 was due to the fragment resulting from the cleavage of ring D.

The ^{1}H-NMR spectrum ($CDCl_3$, 400 MHz) showed two 1H AB doublets at δ 0.60 and 0.65 (J= 4.9 Hz) due to the C-19 cyclopropyl methylene protons. Appearance of both cyclopropyl protons in the ^{1}H-NMR spectrum indicated the absence of oxygen and C=C fuctionalities at C-1 and C-11. Four methyl singlets were observed at δ 0.86, 1.15, 1.37 and 1.42 and a two-proton multiplet at δ 2.68 was ascribed to the C-2 methylene protons. The multiplets at δ 2.20 and 2.40 were assigned to the C-16 methylene. The ^{13}C-NMR spectrum of (+)-buxaheptalactone (**2**) showed all 22 carbon atoms of which the peaks at δ 221.0 and 175.0 were of a cyclopentyl ketone and seven-membered lactone carbonyl, respectively. The peaks due to the four methyl resonances appeared at δ 19.83, 20.62, 22.98 and 30.96 and were assigned to the C-32, C-18, C-30 and C-31 atoms, respectively. The multiplicities of the carbon signals were determined from a DEPT and a broad-band decoupled ^{13}C-NMR spectrum and are presented in Table 1. The carbon-hydrogen connectivities were confirmed by a hetero-COSY experiment.

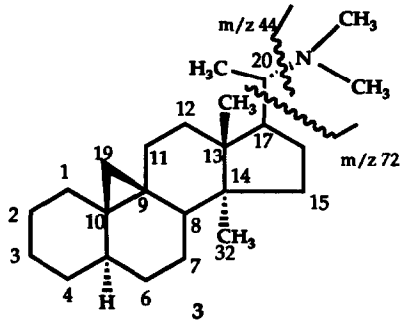


2

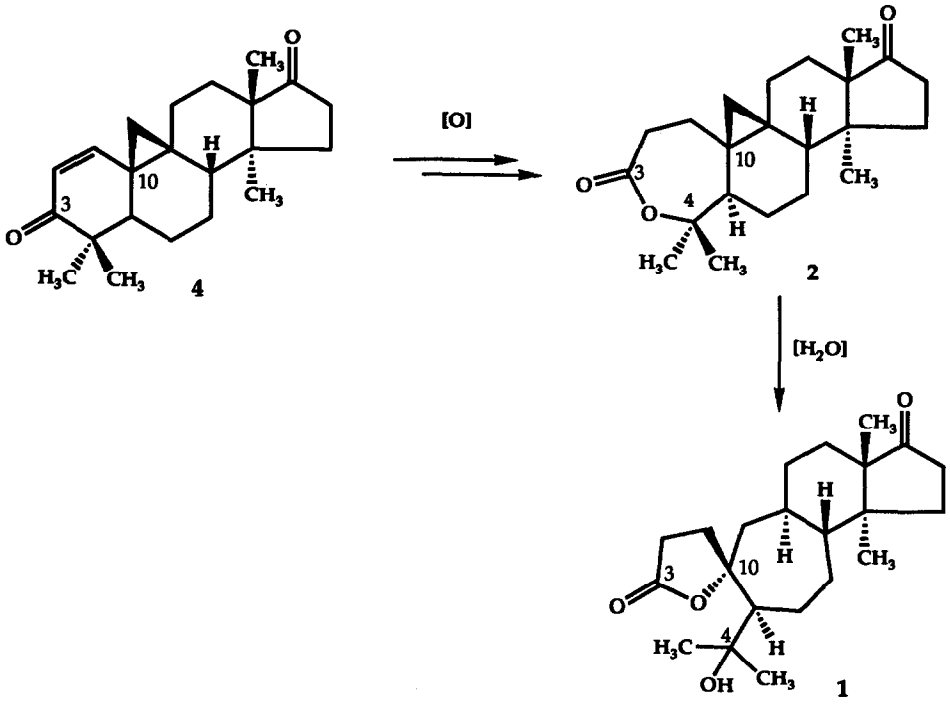
(+)-3-Deoxybuxandonine (**3**) was obtained as a colorless amorphous powder at pH 9. The mass spectrum of **3** revealed the molecular ion (M^+) peak at *m/z* 343.3238, corresponding to the molecular formula $C_{24}H_{41}N$ (calcd. 343.3234). The compound showed only terminal absorption in its UV spectrum. Bands were present at 2910 (C-H) and 1100 (N-C) cm^{-1} in the IR spectrum. The ^{1}H-NMR spectrum was particularly informative, showing only two methyl singlets at δ 0.91 and the 0.96 for C-31 and C-32 protons. Lack of methyl singlets below δ 1.0 indicated the 4,4-bisnor nature of ring A. Another 3H doublet at δ 1.32 (J= 5.8 Hz) was assigned to the secondary C-21 methyl protons and a six-proton broad singlet at δ 2.29 for the *N,N*-dimethylamino substituent at C-20 of the ring D side chain. Two AB doublets at δ 0.30 and 0.54 (J= 4.0 Hz) were assigned to C-19 α and β protons. In the ^{13}C-NMR spectrum, the peaks at δ 16.0 and 18.4 were assigned to the two tertiary methyl carbons (C-31 and C-32), while the *N,N*-dimethylamino carbons appeared at δ 40.0. The multiplicities of various carbons were confirmed by DEPT and broad-band experiments. Of the 24 carbons observed, five were CH_3, eleven were CH_2, four were CH and hence there were four quaternary carbons.

The mass spectrum of **3** showed M^+ at *m/z* 343.3234. A peak at *m/z* 328 resulted from the loss of a methyl group from the molecular ion while the base peak at *m/z* 72.0813 was

due to the cleavage of trimethyliminium ion from ring D. The major fragmentation points are presented around structure **3**. The above studies led to structure **3** for this new steroidal base, trivially named as 3-deoxybuxandonine.



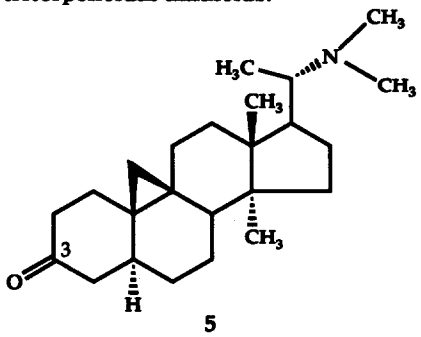
Our chemical studies of *Buxus papillosa* roots have shown it to be a very rich source of new triterpenoids. While the exact biogenetic origins are not yet known, Scheme 1 outlines a potential biogenetic relationship that could exist between **1**,**2** and **4**.



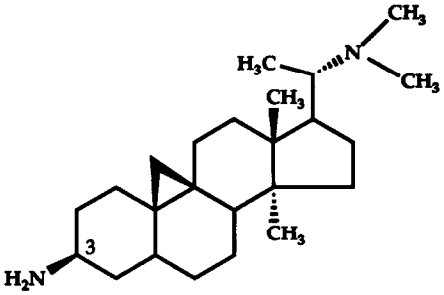
Scheme 1

Scheme 1: Proposed biogenetic relationships for compounds **1**, **2** and **4**.

Compound **3**, in contrast, may arise in nature by the complete reduction of (+)-buxandonine-E (**5**)[12] or by the reductive deamination of cyclobuxargentine-G (**6**).[13] Compounds **3**, **5** and **6** represent a unique group of *Buxus* bases biogenetically situated between true steroidal and triterpenoidal alkaloids.



5



6

Table 1: ^{13}C- and ^{1}H-NMR Data of **1** and **2** in $CDCl_3$.

	1		**2**	
Carbon No.	^{13}C-NMR,δ,m*	^{1}H-NMR,δ,J(Hz)	^{13}C-NMR,δ,m*	^{1}H-NMR,δ,J(Hz) δ,
1	29.7, t	2.65	25.6, t	2.68
2	28.8, t	1.75	34.9, t	0.70
		3.02dt, 9.5, 13.4		1.82
3	177.0, s	-	175.0, s	-
4	73.9, s	-	86.0, s	-
5	47.1, d	1.52	49.4, d	-
6	31.8, t	1.29	26.4, t	1.25
		1.90		2.05
7	25.4, t	1.39	23.8, t	1.45
		1.59		1.10
8	47.1, d	0.90	47.5, d	1.55
9	55.0, d	1.90	22.8, s	-
10	91.9, s	-	27.5, s	-
11	25.2, t	1.29	24.4*, t	1.75
		1.68		1.50
12	30.4, t	1.58	30.0*, t	1.80
		1.61		1.53
13	53.0, s	-	52.0, s	-
14	43.9, s	-	43.9, s	-
15	30.7, t	1.62	32.2, t	1.72
		1.74		1.71
16	33.7, t	2.19dt, 8.7, 19.1	34.1, t	2.20dt, 9.2, 19.4
		2.39ddd, 1.2, 9.6, 19.1		2.40ddd, 3.3,8.4, 19.4
17	219.0, s	-	221.0, s	-
18	18.3, q	0.83s	20.6, q	1.15s
19	51.6, t	1.62	29.4, t	0.60d, 4.9
		1.90		0.70d, 4.9
30	28.4, q	1.27s	31.0, q	1.40s
31	31.7, q	1.29s	23.0, q	1.45s
32	16.6, q	0.99s	19.8, q	0.90s

m* = multiplicity

Acknowledgement: The authors are grateful to ISESCO, Rabat, Morocco for the travel support to M. I. C. Work at Cornell was partially supported by NIH grant.

Experimental

General: Mass spectra were recorded on a Varian MAT 112S mass spectrometer connected to a DEC PDP 11/34 computer system. HRMS were recorded on a JEOL JMS-HX110 mass spectrometer. ^{1}H-NMR were recorded in $CDCl_3$ on Bruker AM-400 instrument at 400 MHz, while ^{13}C-NMR spectra recorded on the same instrument at 100 MHz. Ultraviolet and infrared spectra were recorded on Shimadzu UV 240 and Shimadzu IR 460 spectrophotometers, respectively. Optical rotations were recorded on a Jasco DIP-360 digital polarimeter. The purity of the samples was checked on TLC (silica gel precoated plates). The plant material was identified by the plant taxonomist in the Department of Botany, University of Karachi, where a voucher specimen of the plant has been deposited.

Isolation of (+)-Buxapentalactone (1) and (+)-Buxaheptalactone (2)

The EtOH extract of *Buxus papillosa* roots (100 kg) was evaporated to a gum and the material was redissolved in water. Fractions were obtained by fractionation with $CHCl_3$ at different pH values. The fraction obtained at pH 3.5 (160 g) was loaded on a silica gel column (70-230 mesh, ASTM, Merck). Elution with $CHCl_3$ afforded a mixture of two triterpenes. This mixture was subjected to medium pressure liquid chromatography using a silica gel column (LiCroprep Si 60, 40-63 μm) employing pet.ether-EtOAc-Et_2NH (6:4:0.1) as the eluting solvent. This afforded compounds **1** and **2**.

(+)- Buxapentalactone (1): Colorless crystals (70 mg); $[\alpha]^{29}_D$ = 10.9° ($CHCl_3$); UV λ_{max} (MeOH) 230 nm; IR (KBr) ν_{max} cm^{-1} : 1090 (C-O), 1730 (cyclopentanone), 1742 (lactone) and 3450 (O-H); ^{1}H-NMR ($CDCl_3$, 400 MHz) Table 1; ^{13}C-NMR ($CDCl_3$, 100 MHz) Table 1; HRMS *m/z* (rel.int., %): 362.2465 ($C_{22}H_{34}O_4$, 20), 347.2215 ($C_{21}H_{31}O_4$, 24), 344 ($C_{22}H_{32}O_3$, 10), 304.2062 ($C_{19}H_{28}O_3$, 100), 271.1718 ($C_{18}H_{23}O_2$, 38).

(+)- Buxaheptalactone (2): Colorless crystals (40 mg); $[\alpha]^{29}_D$ = 70° ($CHCl_3$); UV λ_{max} (MeOH) 230 nm; IR (KBr) ν_{max} cm^{-1} : 1000 (C-O), 1710 (ester carbonyl), 1730 (cyclopentanone); ^{1}H-NMR ($CDCl_3$, 400 MHz) Table 2; ^{13}C-NMR ($CDCl_3$, 100 MHz) Table 1; HRMS *m/z* (rel.int., %): 344.2356 ($C_{22}H_{32}O_3$, 100), 329.2126 ($C_{21}H_{29}O_3$, 85), 287.1954 ($C_{19}H_{27}O_2$,12), 231.1747 ($C_{16}H_{23}O_2$, 29), 111.0800 ($C_7H_{11}O_2$,19).

Isolation of (+)-3-Deoxybuxandonine (3)

The ethanolic extract of roots was evaporated to a gum (500 gm) and resuspended in aqueous

different pH values. The fraction obtained at pH 9.0 (60 gm) was loaded on a silica gel column (300 g silica gel). Elution was carried out first with hexane and then with $CHCl_3$-MeOH mixtures. The fraction collected on elution with $CHCl_3$-MeOH (9:1) was washed repeatedly with acetone to supply amorphous **3** (40 mg); $[\alpha]^{26}_D = 10^o$ ($CHCl_3$); UV λ_{max} (MeOH): 201, 264, 275 nm; IR ν_{max} ($CHCl_3$) cm^{-1} : 1100 (C-N); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ : 0.30 (1H, d, J = 4.0 Hz, H-19α), 0.54 (1H, d, J = 4.0 Hz, H-19), 0.91 (3H, s, Me), 0.96 (3H, s, Me), 1.32 (3H, d, J = 5.8 Hz, CH_3-21), 2.29 (6H, s, $N(Me)_2$); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 29.8 (C-1), 32.7 (C-2), 26.4 (C-3), 35.4 (C-4), 44.4 (C-5), 27.4 (C-6), 21.5 (C-7), 48.3 (C-8), 19.4 (C-9), 26.1 (C-10), 26.1 (C-11), 35.5 (C-12), 41.9 (C-13), 49.0 (C-14), 32.7 (C-15), 20.5 (C-16), 49.4 (C-17), 18.3 (C-18), 33.6 (C-19), 50.7 (C-20), 9.3 (C-21), 15.98 (C-32), 40.0 ($N(CH_3)_2$); MS *m/z* (rel.int., %): 343.3288 ($C_{24}H_{41}N$, 10), 328 ($C_{23}H_{38}N$, 18), 298 ($C_{22}H_{35}$, 2), 85 ($C_5H_{11}N$, 22), 72.0813 ($C_4H_{10}N$, 100).

REFERENCES

1. On leave from H. E. J. Research Institute of Chemistry, University of Karachi, Karachi-75270, Pakistan.
2. Choudhary, M. I; Atta-ur-Rahman ; Freyer, A. J; Shamma, M. *Tetrahedron*,**1986**, *42*, 5747.
3. "Structural Studies on New Steroidal Alkaloids of *Buxus papillosa*", Atta-ur-Rahman and M. I. Choudhary, In *New Trends in Natural Products Chemistry*, Atta-ur-Rahman, Ed., Vol. 2, Elsevier Science Publishers, Amsterdam, p. 175, 1988.
4. Steroidal Alkaloids of Apocynaceae and Buxaceae", Atta-ur-Rahman and Muzaffar, A, In *The Alkaloids*, A. Brossi, Ed., Vol. 32, Academic Press, Inc., California, p. 79 (1988).
5. Atta-ur-Rahman; Nasir, H; Iqbal, Z; Choudhary, M. I; Alam, M. *Phytochemistry*, **1989**, *28*, 2848.
6. Pavia, D. L; Lampman, G. M; Kris,G. S. *Introduction to Spectroscopy*,. Saunders College Publishing, Philadalphia, p. 57, 1979.
7. Sheldrick, R. G. *SHELXTL PLUS, Structure Solving Package*, Nicolet, Madison, U. S. A , 1986.
8. Archival X-ray crystallographic data have been deposited and can be ordered from the Cambridge Crystallographic Data Center, University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1 EW, U. K. (please provide complete reference when ordering).
9. Allinger, N. L. *Adv. Phys. Org. Chem.*, **1976**, 13, 1.
10. Atta-ur-Rahman, *One and Two Dimensional NMR Spectroscopy*, Elsevier Science Publishers, Amsterdam , 1989.
11. Atta-ur-Rahman, *Nuclear Magnetic Resonance Spectroscopy*, Springer-Verlag , 1986.
12. Dopke. W; B. Muller. *Pharmazie* **1967**, *22*, 666.
13. Kuchklova, K. I; Voticky, Z; Paulik. V. *Chem. Zvesti* **1976**, *30*,174.