CONTRIBUTION TO THE STEREOCHEMISTRY OF CYCLOBUXAMINE*

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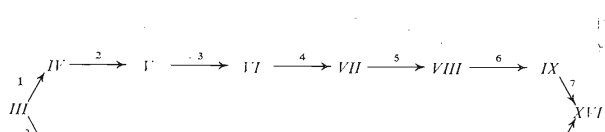
The 4α- and 4β-methyl-N-chlorodihydro derivatives of cyclobuxine-C were synthesized from cyclobuxine-D. Both stereoisomers afforded by Ruschig reaction (20*S*)-4α,14α-dimethyl-20-dimethylamino-16α-hydroxy-9β,19-cyclo-5α-pregnan-3-one, as a result of the epimerization of the axial methyl group of the $C_{(4)}$β-methyl derivative during the degradation. The orientation of the $C_{(4)}$-methyl group of the dihydro derivative, prepared by a catalytic hydrogenation of cyclobuxine-D, was verified on the basis of ^{1}H-NMR evidence. Consequently, the configuration of the $C_{(4)}$-methyl group in cyclobuxamine (dihydrocyclobuxine) was inferred improperly and hence this alkaloid does not constitute any exception and belongs to 4α-methylcyclosteroids.

The constitution and configuration of cyclobuxamine-H** (dihydrocyclobuxine-H) (*I*) was deduced on the basis of indirect arguments; it has been pointed out that it is the first cyclosteroid isolated from a natural material having an axially oriented methyl group at $C_{(4)}$ (ref.[2]). The methyl group at $C_{(4)}$ of synthetic derivatives was originally assigned an equatorial, *i.e.* α-configuration in accordance with a positive Cotton effect of the diketone *II* prepared by a hydrogenation and Ruschig degradation[3] of cyclobuxine-D (*III*) (ref.[4]). Comparison of the ^{1}H-NMR spectra of the native buxus alkaloids possessing one methyl group at $C_{(4)}$ with those of methyl derivatives prepared by a catalytic hydrogenation of the $C_{(4)}$-exomethylene group showed that the doublets of cyclopropyl methylene protons of the former were approximately by 0·20 and 0·29 ppm upfield shifted as compared with the synthetic dihydro derivatives[5]. The intramolecular interaction of the $C_{(4)}$-methyl group in an axial arrangement exerts a deshielding effect on the opposite cyclopropyl methylene protons[6] and therefore, the paramagnetic shift indicated quite the reverse β-configuration of the $C_{(4)}$-methyl group of the synthetic derivatives. It is obvious that epimerization had to take place during the Ruschig degradation. This presumption was evidenced by conversion of both $C_{(4)}$α-(*IX*) and $C_{(4)}$β-(*XV*) N-chloro derivatives into the ketone *XVI* by a reaction sequence given in the Scheme 1.

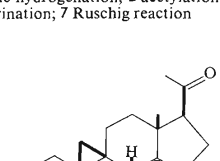
* Part XV in the series *Buxus* Alkaloids; Part XIV: Chem. Zvesti, in press.

** The letter suffixes designate, according to the convention adopted in Kyoto[1], the substitution pattern of methyl groups at nitrogen atoms.

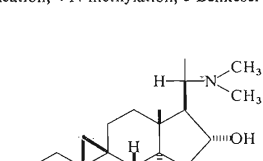


SCHEME 1

1 Catalytic hydrogenation; 2 acetylation; 3 saponification; 4 N-methylation; 5 Benkeser reaction; 6 N-chlorination; 7 Ruschig reaction



II

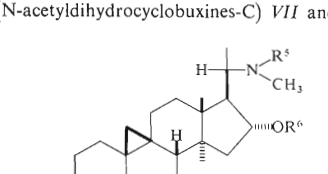


XVI

The $C_{(4)}$-epimers of dihydrocyclobuxine-D can be stereoselectively synthesized in virtually quantitative yields. The catalytic hydrogenation of cyclobuxine-D over Adams catalyst in acetic acid afforded a dihydro derivative the cyclopropyl methylene protons of which resonated at $\delta = 0{\cdot}27$ and $0{\cdot}57$ ppm. Since the position of the AB quartet, associated with cyclopropyl methylene protons of naturally occurring $C_{(4)}$-monomethyl derivatives of cyclosteroids is in the $\delta = 0{\cdot}09-0{\cdot}18$ and $0{\cdot}33-0{\cdot}50$ ppm ranges[5], this isomer had to exhibit an opposite configuration and we ascribed it the structure *IV*. The signal of the $C_{(4)}$-methyl group is not decisive for the configuration, as there is no significant difference between chemical shifts in both series[7].

The catalytic hydrogenation of N,N′,O-triacetylcyclobuxine-D (*X*), prepared by acetylation of cyclobuxine-D, led unambiguously to the $C_{(4)}$-epimer *XI* since the specific rotation, m.p. and positions of the cyclopropyl methylene protons in the ^{1}H-NMR spectrum considerably differ from those of *V* (*cf.*[2]). Further synthetic route was identical in both series: hydrolysis of N,N′,O-triacetyl derivatives *V* and *XI* afforded amides *VI* and *XII*. The IR spectrum of N-acetyldihydrocyclobuxine-D (*XII*) showed bands at 1312, 1410 and 1647 cm^{-1} (amide I, II, III), 1018 and 3430 cm^{-1} (a hydroxyl group) and 3310 cm^{-1} (sec-amine); the mass spectrum revealed the peak of molecular radical ion at *m/e* 430 and further prominent peaks at *m/e* 415 (M − 15), 373 (M − 57), 58 (base peak) and 43; the ^{1}H-NMR spectrum exhibited signals

of cyclopropyl methylene protons (on the δ scale in p.p.m.) at 0·16 and 0·39 (2 H, dd, J = 4 Hz), $C_{(4)}$-sec-methyl group at 0·80 (3 H, d, J = 6 Hz), $C_{(18)}$-tert-methyl group at 0·97 (3 H, s), $C_{(14)}$-tert-methyl group at 1·10 (3 H, s), $C_{(21)}$-sec-methyl group at 1·08 (3 H, d, J = 6·5 Hz), methyl of the acetyl group at 2·08 (3 H, s), N′-methylamino group at 2·43 (3 H, s), N-methyl group at 2·74 (3 H, s) and a $C_{(16)}$ proton at 4·09 (1 H, m). Amides *VI* and *XII* gave on methylation N-acetyl-N′-methyldihydrocyclobuxines-D (N-acetyldihydrocyclobuxines-C) *VII* and *XIII*; their mass



I, $R^1 = R^2 = R^3 = H$, $R^4 = CH_3$, $R^5 = R^6 = H$
III, $R^1 = H$, $R^2 = CH_3$, $R^3, R^4 = CH_2$, $R^5 = R^6 = H$
IV, $R^1 = H$, $R^2 = CH_3$, $R^3 = H$, $R^4 = CH_3$, $R^5 = R^6 = H$
V, $R^1 = Ac$, $R^2 = CH_3$, $R^3 = H$, $R^4 = CH_3$, $R^5 = R^6 = Ac$
VI, $R^1 = Ac$, $R^2 = CH_3$, $R^3 = H$, $R^4 = CH_3$, $R^5 = R^6 = H$
VII, $R^1 = Ac$, $R^2 = CH_3$, $R^3 = H$, $R^4 = R^5 = CH_3$, $R^6 = H$
VIII, $R^1 = H$, $R^2 = CH_3$, $R^3 = H$, $R^4 = R^5 = CH_3$, $R^6 = H$
IX, $R^1 = Cl$, $R^2 = CH_3$, $R^3 = H$, $R^4 = R^5 = CH_3$, $R^6 = H$
X, $R^1 = Ac$, $R^2 = CH_3$, $R^3, R^4 = CH_2$, $R^5 = R^6 = Ac$
XI, $R^1 = Ac$, $R^2 = R^3 = CH_3$, $R^4 = H$, $R^5 = R^6 = Ac$
XII, $R^1 = Ac$, $R^2 = R^3 = CH_3$, $R^4 = R^5 = R^6 = H$
XIII, $R^1 = Ac$, $R^2 = R^3 = CH_3$, $R^4 = H$, $R^5 = CH_3$, $R^6 = H$
XIV, $R^1 = H$, $R^2 = R^3 = CH_3$, $R^4 = H$, $R^5 = CH_3$, $R^6 = H$
XV, $R^1 = Cl$, $R^2 = R^3 = CH_3$, $R^4 = H$, $R^5 = CH_3$, $R^6 = H$
XVII, $R^1 = R^2 = H$, $R^3 = CH_3$, $R^4 = R^5 = R^6 = H$

spectra showed, in addition to the molecular radical ion at m/e 444, peaks at m/e 429, 411, 400, 356 and 72 (base peak). Bands in the IR spectrum at 1250 and 2788 cm^{-1} were characteristic of a tert-amine, whilst the band at 3310 cm^{-1} was lacking. The signals of cyclopropyl methylene protons in the ^{1}H-NMR spectrum of compound *VII* were at 0·33 and 0·58 (2 H, dd, J = 4 Hz), those of *XIII* at 0·07 and 0·30 (2 H, dd, J = 4 Hz). Amides *VI* and *XII* resisted the drastic conditions on alkaline hydrolysis and therefore the Benkeser reaction[8] was employed for deacetylation of substances *VII* and *XIII*. The enantiomer with equatorially oriented methyl group *XIV* had peaks in the mass spectrum at m/e 402 (M^+) and a fragmentation pattern characteristic of an N-methylamino-N′-dimethylamino substitution on the steroidal backbone[9]. The band of a sec-amino group at 3330 cm^{-1} occured in the IR spectrum

instead of vibrations belonging to an amide. The ^{1}H-NMR spectra of the pair of amines *VIII* and *XIV* again differed mainly in the position of signals of cyclopropyl methylene protons: with amine *VIII* they appeared at 0·30 and 0·59 (2 H, dd, J = 4 Hz) with amine *XIV* at 0·12 and 0·49 (2 H, dd, J = 4 Hz). The N-chloro derivatives *IX* and *XV*, needed for Ruschig degradation, were synthesized from compounds *VIII* and *XIV* and N-chlorosuccinimide. The mass spectra of both N-chlorodihydrocyclobuxines-C did not contain the peak of molecular radical ion. The parent peak at m/e 72 was associated with the ion generated by a cleavage of the dimethylamino radical from the ionized molecule. The fragmentation was triggered by the fission of hydrogen chloride and continued as described earlier[9]. The IR spectra of both derivatives were virtually identical. The difference in signal positions in the ^{1}H-NMR spectra are seen in Table I.

Chloramines *IX* and *XV* furnished upon Ruschig degradation a single ketone *XVI*. Its mass spectrum displayed peaks at m/e 72 (base peak), 372 (M − 15) and 387 (M^+), the IR spectrum bands attributable to vibrations of a hydroxyl group (1035 and 3420 cm^{-1}), a cyclopropyl methylene (1475 and 3045 cm^{-1}) and a ketone (1715 cm^{-1}). The UV spectrum was indicative of an isolated carbonyl (282 nm, log ε 1·78). The ORD spectrum showed a positive Cotton effect ($[\Phi]_{303}$ +2700° and $[\Phi]_{266}$ −2700°) typical of 4α-methyl-3-keto-9β,19-cyclo-5α-steroids[10]. Signals of protons in the ^{1}H-NMR spectrum were found to be in accordance with those reported[11]. The position of signals of cyclopropyl methylene protons was influenced by the anizotropic effect of the $C_{(3)}$-carbonyl group[6], interacting with the neighbouring

TABLE I

Chemical Shifts (δ, ppm) of $C_{(4)}$-Epimeric N-Chlorodihydrocyclobuxines-C

Proton	Number	Multiplicity	J, Hz	*IX*	*XV*
Cyclopropylmethylene	1	d	4·5	0·29	0·13
Cyclopropylmethylene	1	d	4·5	0·58	0·39
$H_3C_{(21)}$	3	d	6·0	0·83	0·89
$C_{(4)}$—CH_3	3	d	6·0	0·89	0·95
$H_3C_{(18)}$	3	s	—	0·96	0·98
$C_{(14)}$—CH_3	3	s	—	1·12	1·13
$(CH_3)_2N$	6	s	—	2·25	2·28
CH_3NH	3	s	—	2·94	2·86
$C_{(20)}$—H	1	q	6·5	3·69	3·72
$C_{(16)}$—H	1	m	—	4·06	4·10

α-methyl group and is therefore not characteristic of the orientation of the methyl group at $C_{(4)}$.

As evident, epimerization of the axially oriented methyl group at $C_{(4)}$ into a sterically more favourable α (equatorial) position took place during the Ruschig degradation. This argument proves that the native 4-methylcyclosteroids, the representative of which is cyclobuxine-H (*XVII*) do not constitute an exception and do belong to the 4α-methylcyclosteroids.

EXPERIMENTAL

The synthesized compounds were dried at 13 Pa and 78°C. The melting points were determined on a Kofler micro hot-stage, optical rotation of chloroform solutions with a Perkin Elmer 141 apparatus in 1 cm cells, mass spectra with an MCh 1306 (USSR) spectrometer adapted for a direct introduction of the sample to the ionization chamber at the ionizing electron energy 70 eV and 1 mA trap current. The IR spectra were recorded with a Perkin-Elmer spectrometer model 457 in KBr discs, the UV spectra and ORD curves of ethanolic solutions with an ORD/UV-5 Jasco instrument. The ^{1}H-NMR spectra of deuteriochloroform solutions were taken with a Tesla 487 B apparatus at 80 MHz (internal reference substance tetramethylsilane). Alumina for chromatography according to Brockmann (Reanal) was of activity grade VI; separation of substances was monitored by loose-layer chromatoplates coated with the same support in the solvent system benzene–chloroform–ethanol (7 : 15 : 3). Cyclobuxine-D was isolated from common box (*Buxus sempervirens* L. ref.[9,12]) and identified by spectral means and mixed m.p. with the authentic specimen.

Derivatives of the 4β-Methyl Series

Dihydrocyclobuxine-D (IV): Cyclobuxine-D (*III*) (2·135 g) was catalytically hydrogenated over Adams catalyst (56 mg) in acetic acid (45 ml) at room temperature. The mixture was worked up to yield 2·17 g of the title product, m.p. 206–208°C (dichloromethane–acetone), $[\alpha]_D^{23}$ +43° (*c* 1·0).

N,N′,O-*Triacetyldihydrocyclobuxine*-D (V): Substance *IV* (2·15 g) was acetylated in pyridine–acetic anhydride (40 ml and 20 ml) for 26 h at room temperature. The reaction mixture was worked up in a usual manner. Yield 2·75 g, m.p. 228–231°C (acetone), $[\alpha]_D^{22}$ −60° (*c* 0·9).

N-*Acetyldihydrocyclobuxine*-D (VI): Compound *V* (2·70 g) was hydrolyzed with a 40% solution of KOH in 80% ethanol (70 ml) for 4 h under a reflux condenser. Ethanol was distilled off *in vacuo*, the residue diluted with water (25 ml) and extracted with dichloromethane. The solvent was evaporated and the residue purified by column chromatography. Yield 1·2 g, m.p. 243–245°C (dichloromethane–acetone), $[\alpha]_D^{23}$ +7° (*c* 0·9).

N-*Acetyl*-N′-*methyldihydrocyclobuxine*-D (N-acetyldihydrocyclobuxine-C) (*VII*): The product *VI* (1·1 g) was dissolved in dichloromethane–acetone (25 ml each), methyl iodide (2 ml) was added and the solution was allowed to stand for 6 days at +3°C. To the mixture concentrated under reduced pressure a 30% methanolic KOH (15 ml) was added, heated at 50°C and finally kept 1 h at room temperature. Methanol was removed *in vacuo*, the solid suspended in water (50 ml) and extracted with dichloromethane. The evaporated extract was purified by column chromatography. Yield 630 mg, m.p. 215–217°C (dichloromethane–acetone), $[\alpha]_D^{24}$ −7° (*c* 1·86).

Dihydrocyclobuxine-C (VIII): The derivative *VII* (600 mg) was suspended in ethylamine (50 ml), cooled to 0°C and lithium metal (300 mg) was portionwise added to this suspension at 0–5°C. The excess of lithium was decomposed with methanol (5 ml) and water (40 ml). The solvent was distilled off under diminished pressure and the product was extracted with dichloromethane. The concentrated extract was purified by column chromatography and preparative thin-layer chromatography. Yield 85 mg, m.p. 253–255°C (dichloromethane–acetone), $[\alpha]_D^{20}$ +28° (*c* 1·2).

N-*Chlorodihydrocyclobuxine*-C (IX): The amine *VIII* (50 mg) was dissolved in dichloromethane (50 ml), cooled to −5°C, N-chlorosuccinimide (40 mg) in dichloromethane (5 ml) was added during 20 min at 0°C and the solution was allowed to stand for additional 30 min. The solution was then washed with water, the organic layer dried and the solvent removed *in vacuo*. Yield 55 mg, m.p. 225°C (dec.) (dichloromethane–acetone), $[\alpha]_D^{27}$ +115° (*c* 0·66). For $C_{26}H_{45}ClN_2O$ (437·2) calculated: 8·1% Cl; found: 8·56% Cl.

Derivatives of the 4α-Methyl Series

N,N',O-*Triacetylcyclobuxine*-D (X): Cyclobuxine-D (*III*) (1·92 g) was acetylated as described in the preparation of *V*. Yield 2·5 g, m.p. 258–259°C (acetone), $[\alpha]_D^{22}$ −12° (*c* 1·2).

N,N',O-*Triacetyldihydrocyclobuxine*-D (XI): Compound *X* (2·4 g) was hydrogenated in acetic acid (50 ml) as *IV*. Yield 2·4 g, m.p. 224–225°C (dichloromethane–acetone), $[\alpha]_D^{20}$ −46° (*c* 1·8).

N-*Acetyldihydrocyclobuxine*-D (XII): The acetate *XI* (2·3 g) was hydrolyzed and worked up as *VI*. Yield 1·1 g, m.p. 238–240°C (dichloromethane–acetone), $[\alpha]_D^{21}$ +18° (*c* 1·4).

N-*Acetyl*-N'-*methyldihydrocyclobuxine*-D (N-acetyldihydrocyclobuxine-C) (*XIII*): The derivative *XII* (1·0 g) was methylated and worked up as *VII*. Yield 510 mg, m.p. 221–224°C (dichloromethane–acetone), $[\alpha]_D^{23}$ +22° (*c* 0·9).

Dihydrocyclobuxine-C (XIV): Product *XIII* (420 mg) was reacted as *VII* to give *VIII*. Yield 70 mg, m.p. 188–190°C (ether–acetone), $[\alpha]_D^{25}$ +37° (*c* 1·4).

N-*Chlorodihydrocyclobuxine*-C (XV): Substance *XIV* (35 mg) was chlorinated as *IX*. Yield 39 mg, m.p. 238°C (dec.), (dichloromethane–acetone), $[\alpha]_D^{24}$ +69° (*c* 1·3). For $C_{26}H_{45}ClN_2O$ (437·2) calculated: 8·1% Cl; found: 8·48% Cl.

(20s)-16α-*Hydroxy*-20-*dimethylamino*-4α,14α-*dimethyl*-9β,19-*cyclo*-5α-*pregnan*-3-*one* (XVI): A solution of *XV* (290 mg) in methanol (5 ml) was poured into a solution of sodium metal (140 mg) in methanol (10 ml) and heated under a reflux condenser for 90 min. Methanol was distilled off under reduced pressure, the residue extracted with dichloromethane, the solvent evaporated and the solid was allowed to stand in a mixture of 1·5M-H_2SO_4 and methanol (10 ml each) for 15 h at room temperature. The mixture was diluted with water, made alkaline with ammonium hydroxide and extracted with dichloromethane. The crude product was purified by a preparative thin-layer chromatography. Yield 180 mg.

N-Chlorodihydrocyclobuxine-C (IX) (45 mg) was degrated in the same way. Yield 22 mg, m.p. 196–198°C (ether), $[\alpha]_D^{25}$ +31° (*c* 1·4). The mixed m.p. of ketones of both series exhibited no depression.

The spectra of the synthesized products and the elemental analyses were carried out in the Department of analytical chemistry (Head Dr C. Peciar).

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