Buxus Alkaloids. X.*
The Occurrence of a *trans* Isomer of Cyclosuffrobuxinine

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From the fraction "L" of the alkaloid mixture of the common box (*Buxus sempervirens* L.) a new alkaloid was isolated and its structure elucidated. It happened to be the first *Buxus* alkaloid characterized by a *trans*-α,β-unsaturated cyclopentenone in its molecule; alkaloids of this kind are believed to be artifacts and consequently, no trivial name has been given to this substance.

From the fraction "L" [1] of the alkaloids of *Buxus sempervirens* L. we were able to isolate a substance yellow in colour and oily in nature which resisted all crystallization attempts. Although thin-layer chromatography on both kieselguhr G and silica gel G showed one spot, the NMR spectrum of this substance was rather complex, suggesting in the first approximation the presence of two series of signals. By repeated chromatography of this base on various carriers (alumina neutral and slightly basic, grade I–VI, Florisil) the separation was not achieved. Finally, it was succeeded to distribute the product into two substances *I* and *II* by partition chromatography similar to that already described [2]. By the separation process the amount of these alkaloids substantially dropped, so that it was decided to take spectra of the oily products without having them crystalline. The *I* : *II* ratio was estimated as being approximately 1 1.

The mass spectra of the compounds *I* and *II* were found to be superimposable: molecular ion peak appearing at *m/e* 353 was followed by peaks at *m/e* 338 (M−15, loss of a methyl radical), 325 and 324 (M−28 and M−29 characteristic of a ketone), 310 (M−43) and fragmentation pattern *m/e* 44, 57, 70 indicative of a monomethylamino grouping at C-3. The low intensity of the peak at *m/e* 70 and to some extent also of that at *m/e* 57 suggests [3] the presence of an exomethylene group at C-4. The ultraviolet spectrum shows a carbonyl conjugated with a double bond (λ_{max} 243 nm, log ε 4.00).

Also the infrared spectra of substances *I* and *II* are virtually identical revealing the presence of a carbonyl on a five-membered ring (1734 and 1732 cm^{-1} respectively) a $\Delta^{17,20}$ double bond (1680 cm^{-1}), an exomethylene (1662 + 898 cm^{-1}) a cyclopropylmethylene (1465 + 3043 cm^{-1}) and a diffuse band at 3350 cm^{-1} (secondary amine).

Significant difference between substances *I* and *II* has been found in their NMR spectra and the values recorded are listed in Table 1.

Chemical shifts listed in Table 1 are in good agreement with those reported for cyclosuffrobuxinine [4] and *cis*- and *trans*-des-*N'*-16-dehydrodihydrocyclobuxine [5]. This

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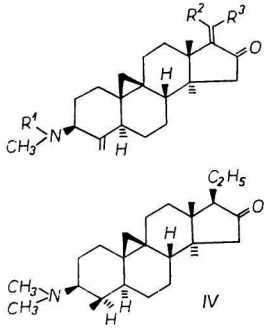
Table 1

Substance *I*		Substance *II*	Assignment
6.44 (q)		5.50 (q)	$=CH \cdot CH_3$
	4.72 (d)		$=CH_2$
2.59		2.13	$-N \cdot CH_3$
1.72 (d)		1.82	$=CH\ CH_3$
1.28		1.21	*tert*-CH_3
			tert-CH_3
1.12		0.93	cyclopropyl
	0.33 + 0.67 (d)		methylene

Chemical shifts are given in p.p.m. on the δ scale, hexamethyldisiloxane being the internal reference; d = doublet, q = quadruplet.

and the other above-mentioned data let us believe the substance *I* to be identical with cyclosuffrobuxinine. Accordingly, the substance *II* should be the *trans* isomer of cyclosuffrobuxinine and consequently, the first *Buxus* alkaloid possessing a *trans*-α,β-unsaturated cyclopentenone configuration in ring D.

Feeling the need for more substantial evidence on the identity of our substance *I* with cyclosuffrobuxinine we prepared both the *N*-methyl derivative (*III*) and the tetrahydro derivative (*IV*) of it. The mass spectrum of the former shows a fragmentation pattern confirming the dimethylamino substitution at C-3 (peaks of lower intensity at *m/e* 58, 71, 84 diagnostic of the exomethylene substitution at C-4), the molecular ion peak at *m/e* 367 and the NMR and ultraviolet spectra almost consistent with those reported for cyclosuffrobuxine (*III*) [4].



IV

Scheme 1

I $R^1 = H$, $R^2 = CH_3$, $R^3 = H$;
II $R^1 = H$, $R^2 = H$, $R^3 = CH_3$;
III $R^1 = CH_3$, $R^2 = CH_3$, $R^3 = H$.

The tetrahydro-*N*-methyl substance *I* (*IV*) displays the fragmentation pattern at *m/e* 71 and 84 about 30 times more intensive than that of the above-mentioned derivative evidencing thus the methyl substitution at C-4; the remaining molecule of hydrogen

saturated the $\Delta^{17,20}$ double bond. In accordance with it both the λ_{max} 243 nm in the ultraviolet and the 1680 cm^{-1} band in the infrared spectra disappeared while that associated with the carbonyl was shifted to 1748 cm^{-1}.

As known, *Buxus* alkaloids, or their derivatives with a C-16 keto group aminated at C-20 rapidly undergo deamination in basic medium or when refluxed in an alcohol to give a mixture of the *cis*- and *trans*-α,β-unsaturated cyclopentenones. This fact has already been pointed out and a hypothesis has been implied that alkaloids of this type described earlier [4] might be artifacts.

To verify this hypothesis, we alkalified the purified extract of *Buxus* alkaloids dissolved in dilute acetic acid with a concentrated aqueous solution of sodium hydroxide in an airtight apparatus at room temperature and while passing pure nitrogen through the mixture, alkaline reacting gases were trapped in dilute hydrochloric acid. Vacuum-dried hydrochlorides of thus obtained volatile bases were identified by means of mass spectrometry and paper chromatography. As anticipated no molecular peak could be found in the mass spectrum; the fragmentation was started by the separation of chlorine to afford the $\overset{(+)}{R}NH_3$ species which is of very low intensity. The next peak in this series, represented by the general formula $\overset{(+)}{R}NH_2$ could be formed by the expulsion of a proton, whereas the loss of another proton would lead to the generation of the parent peak of formula $CH_2{=}\overset{(+)}{N}H_2$ in that case when at least one methyl group was present in the amine molecule. Thus peaks at *m/e* 32, 31, and 30 characteristic of methylamine hydrochloride and those at *m/e* 18, 17, and 16 indicative of ammonium chloride together with peaks due to ^{35}Cl, $H^{35}Cl$, ^{37}Cl, and $H^{37}Cl$ were found in the spectrum, while those at *m/e* 46, 45, and 44 attributable to dimethylamine hydrochloride were not. The absence of this series of fragments might be due to either the minute amount of precursor alkaloids with the dimethylamino substitution at C-20, or the reluctance of those to deaminate.

Further evidence has been brought forward by paper chromatography. Both the mixture of volatile bases as hydrochlorides and the reference substances and their mixture were investigated and the R_F values of the well resolved spots, visualized with silver nitrate solution, were found to be 0.12 and 0.19 for ammonium chloride and methylamine hydrochloride, respectively. As no other nitrogen-containing substances were present in the extract of *Buxus* alkaloids one can suppose that the bases trapped after alkalinization were released from the alkaloids present as a result of deamination taking place at C-20.

The occurrence of the *cis* and *trans* isomers in the extract under the given reaction conditions on one hand and the formation of ammonia and methylamine on the other would mean that these isomers are artifacts. This is the reason why no trivial name has been given to our substance *II*.

It is worth noting that buxazidine-B [6] is the only representative of the series of *Buxus* alkaloids having the methylamino grouping at C-20 and a C-16 carbonyl at the same time; its structure, however, was inferred on the basis of spectral data and has not been confirmed as yet.

So far, there have been reported eight structures of alkaloids isolated from various species of the box family having the structure of a *cis*-α,β-unsaturated cyclopentenone steroid. They are as follows: cyclobuxophyllinine [4] (cyclobuxophyllinine-M [7], buxenone [8]), buxarine [9], cyclosuffrobuxinine, cyclosuffrobuxine, cyclobuxosuffrine, cyclobuxophylline [4], buxene and *N*-methylbuxene [10].

It is our belief that all the above-mentioned substances are artifacts formed during the separation process.

Experimental

Optical rotations were measured in microcell (2 dm) on a Zeiss—Winkel polarimeter (calibration 0.02°) and on a Bendix—Ericsson objective polarimeter in chloroform. Mass spectra were taken with a MCh 1306 spectrometer (USSR) adapted for a direct introduction of the sample to the ionization chamber at the ionizing electron energy 70 eV and 1 mA intensity. The temperature in the vapourization locus was kept constant within the 30—60°C range depending on the volatility of samples. The temperature in the ionization chamber varied from 100—120°C and in single measurement was kept stable. Infrared spectra were recorded with a UR-10 Zeiss spectrophotometer in carbon tetrachloride, NMR spectra were run with a Tesla BS 487B apparatus at 80 MHz, hexamethyldisiloxane being the internal reference, and ultraviolet spectra were measured with an ORD/UV-5 Jasco apparatus in ethanol.

Deamination of the extract

The acetic acid acidified and purified aqueous extract of the leaves of *Buxus sempervirens* L. obtained as described in our previous paper [1] (1000 ml) was made alkaline to pH 14 with a 33% aqueous sodium hydroxide solution in a three-necked round-bottomed flask equipped with an inlet tube, mechanical stirrer and a washing bottle filled with 25 ml of 2% hydrochloric acid. Nitrogen was passed at room temperature through the mixture to expulse volatile bases freed by alkalinization. During the first 1 1/2 hours 70 mg of base hydrochlorides were trapped and during additional 4 and 6 hours the respective 105 and 108 mg of hydrochlorides were obtained after evaporation *in vacuo*.

Chromatography of the base hydrochlorides

The mixture of base hydrochlorides was chromatographed on a Whatman No. 4 sheet together with the respective ammonium chloride, methylamine hydrochloride, dimethylamine hydrochloride and their artificial mixture as reference substances. Good separation was achieved in a system acetic acid—*n*-butanol 25 75 and 5 hours of development using the descending technique. Visualization was made with 0.25 M-$AgNO_3$ after exposure in ultraviolet light. The R_F value of dimethylamine hydrochloride was found to be 0.24, the remaining R_F values were mentioned earlier.

Cyclosuffrobuxinine (substance *I*), *N*-methylcyclosuffrobuxinine (cyclosuffrobuxine) and tetrahydrocyclosuffrobuxine as prepared in our Laboratory were determined to have the physicochemical constants in accordance with those reported.

Substance *II* (*trans*-cyclosuffrobuxinine) $[\alpha]_D^{22}$ −47° (*c* 0.60, $CHCl_3$).

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