water and extraction with methylene chloride gave 7.67 g. of pink solid, m.p. 200–210°. The solid was taken up in methylene chloride and chromatographed on 230 g. of neutral alumina (Woelm, activity grade II–III). Elution with methylene chloride gave a yellow-white solid, m.p. 195–200°, which was crystallized first from methanol and then from isopropyl alcohol to give 0.46 g. of white crystals: m.p. 205–207°; $[\alpha]_D$ −14.1°; ν_{max}^{Nujol} (cm.$^{-1}$) 3410, 2725, 1720, 1677, 1630, 1262, 1240, and 1158; $\nu_{max}^{CHCl_3}$ (cm.$^{-1}$) 3453, 3017, 2957, 2867, 2789, 1719, 1639, 1250, and 1160.

Anal. Calcd. for $C_{23}H_{28}N_2O_4$ (396.47): C, 69.67; H, 7.12; N, 7.07. Found: C, 70.00; H, 7.08; N, 6.96.

Elution of the alumina column with methylene chloride containing 1% methanol gave 2.82 g. of cream-colored solid, m.p. 215–221°. Two crystallizations from methanol gave 1.13 g. of white prisms of IV (R′ = CH_3): m.p. 232–235°; $[\alpha]_D$ +41.0°; ν_{max}^{Nujol} (cm.$^{-1}$) 3387, 1745, 1627, 1274, 1259, 1172, 1145, and 1088; $\nu_{max}^{CHCl_3}$ (cm.$^{-1}$) 3470, 3378, 2920, 2814, 2760, 1735, 1630, 1260, 1150, 1110, 1092 (sh), and 1082.

Anal. Calcd. for $C_{24}H_{32}N_2O_5 \cdot CH_3OH$ (460.58): C, 65.20; H, 7.88; N, 6.08. Found: C, 65.36; H, 7.78; N, 6.22.

The methanol solvate was not completely solvent free even after drying at 140° (0.3 mm.) for 5 hr.

The hydrochloride salt of IV (R′ = CH_3), prepared in acetone by addition of concentrated hydrochloric acid, had m.p. 275–276°; $[\alpha]_D$ +20.6° (MeOH); ν_{max}^{Nujol} (cm.$^{-1}$) 3185, 2669, 2570, 1731, 1632, 1261, 1241, 1153, 1114, 1094 (sh), and 1087.

Anal. Calcd. for $C_{24}H_{32}N_2O_4 \cdot HCl \cdot H_2O$ (467.02): C, 61.72; H, 7.55; N, 6.00. Found: C, 61.57; H, 7.10; N, 5.80.

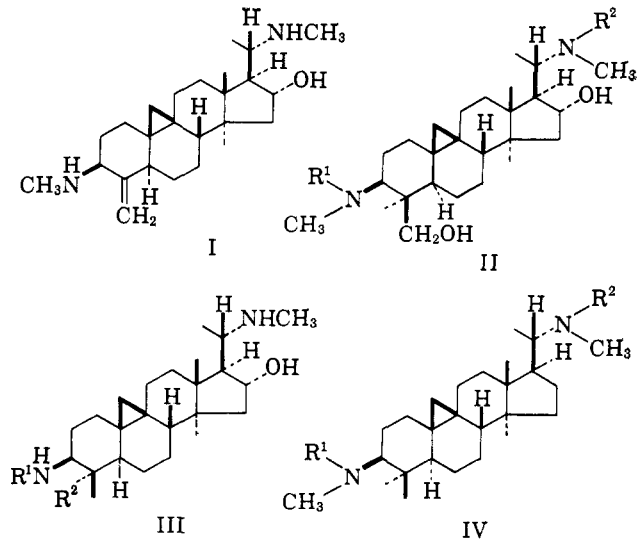
Buxus Alkaloids. VIII. The Isolation and Constitution of Cycloprotobuxine-D*,[1,2]

S. MORRIS KUPCHAN AND E. KUROSAWA

Department of Pharmaceutical Chemistry, University of Wisconsin, Madison 6, Wisconsin

Received November 5, 1964

In 1962 we reported the elucidation of the structure[3] and configuration[4] of cyclobuxine-D (I), an alkaloid isolated from *Buxus sempervirens* L.[5] Cyclobuxine-D was shown to be the prototype of a new class of steroidal alkaloids which contains a cyclopropane ring and which has a substitution pattern at C-4 and C-14 which is intermediate in the biogenetic scheme between lanosterol- and cholesterol-type steroids. Subsequent studies have characterized the following structurally related alkaloids: cyclomicrophylline-A (II, $R^1 = R^2 = CH_3$)[6]; cyclomicrophylline-B (II, $R^1 = CH_3$, $R^2 = H$)[6,7]; cyclomicrophylline-C (II, $R^1 = H$, $R^2 = CH_3$)[6]; cyclobuxamine-H (III, $R^1 = R^2 = H$)[8]; cyclovirobuxine-D (III, $R^1 = R^2 = CH_3$)[9]; and cycloprotobuxine-C (IV, $R^1 = H$, $R^2 = CH_3$).[10] In addition, several new alkaloids containing a novel B-homosteroidal diene system have recently been isolated from *Buxus sempervirens* L.[1,11,12] The isolation from *Buxus sempervirens* L. and elucidation of the structure of an additional new alkaloid, cycloprotobuxine-D (IV, $R^1 = R^2 = H$), is described in the present report.



Cyclobuxine-D was isolated from the acetone-soluble portion of the strong bases obtained by the fractionation procedure described earlier.[3b] Partition chromatography[13] yielded cycloprotobuxine-D, along with cycloprotobuxine-C[10] ("alkaloid L'"[14,15]), buxenine-G,[1] and cyclovirobuxine-D.[9] Cycloprotobuxine-D, $C_{26}H_{46}N_2$, m.p. 140–142°, $[\alpha]^{28}_D$ + 112° (chloroform), has an n.m.r. spectrum showing the presence of two N-methyl groups (τ 7.58, 3H; 7.64, 3H); one secondary C-methyl group (τ 8.96, doublet, J = 6 c.p.s.); four tertiary C-methyl groups (τ 9.02, 9.05, 9.08, and 9.25); and a cyclopropyl methylene (τ 9.45 and 9.70, AB doublets, J = 4.5 c.p.s.). Acetylation with acetic anhydride in pyridine yielded N,N-diacetylcycloprotobuxine-D (IV, $R^1 = R^2 = COCH_3$), which demonstrated an infrared band for two tertiary amide (6.18 μ, very strong) functions and n.m.r. peaks for two N-*methyl*acetamide functions (τ 7.08, 3H; 7.18, 3H; *cf.* ref. 3b); two N-acetyl groups (τ 7.88, 3H; 7.95, 3H); one secondary (τ 8.85, 3H, doublet, J = 6 c.p.s.) and four tertiary C-methyl groups (τ 8.90, 3H; 9.02, 3H; 9.07, 3H; 9.13, 3H); and a cyclopropyl methylene (τ 9.42 and 9.63, AB doublets, J = 4 c.p.s.). The similarity of the infrared spectrum of cycloprotobuxine-D to that of cyclovirobuxine-D (III, $R^1 = R^2 = CH_3$)[9] (other than in the hydroxyl region), and the indication from the foregoing n.m.r. data that the alkaloid is a di(monomethylamino) derivative of a system containing one secondary and four tertiary C-methyl groups and a cyclopropyl methylene group, led to the preliminary formulation of cycloprotobuxine-D as desoxycyclovirobuxine-D.

Strong support for assignment of constitution IV, $R^1 = R^2 = H$, to cycloprotobuxine-D was adduced by

* To Professor Louis F. Fieser.

(1) Part VII: S. M. Kupchan and W. L. Asbun, *Tetrahedron Letters*, 3145 (1964).

(2) This investigation was supported in part by research grants from the National Institutes of Health (CA-04500 and HE-02952).

(3) (a) K. S. Brown, Jr., and S. M. Kupchan, *J. Am. Chem. Soc.*, **84**, 4590 (1962); (b) *ibid.*, **86**, 4414 (1964).

(4) K. S. Brown, Jr., and S. M. Kupchan, *ibid.*, **84**, 4592 (1962); **86**, 4424 (1964).

(5) K. Heusler and E. Schlittler, *Helv. Chim. Acta*, **32**, 2226 (1949).

(6) T. Nakano and S. Terao, *Tetrahedron Letters*, 1035, 1045 (1964).

(7) D. Gautier, F. Khuong-Hui-Laime, E. Stanislas, and R. Goutarel, paper presented to the International Symposium on the Chemistry of Natural Produets, Kyoto, April 1964.

(8) K. S. Brown, Jr., and S. M. Kupchan, *J. Am. Chem. Soc.*, **86**, 4430 (1964).

(9) K. S. Brown, Jr., and S. M. Kupchan, *Tetrahedron Letters*, 2895 (1964). The convention on use of letter suffixes to designate substitution pattern at C-3 and C-20 nitrogen functions is described in this reference.

(10) J. P. Calame and D. Arigoni, *Chimia* (Aarau), **18**, 185 (1964).

(11) W. L. Asbun, *Dissertation Abstr.*, **24**, 4415 (1964).

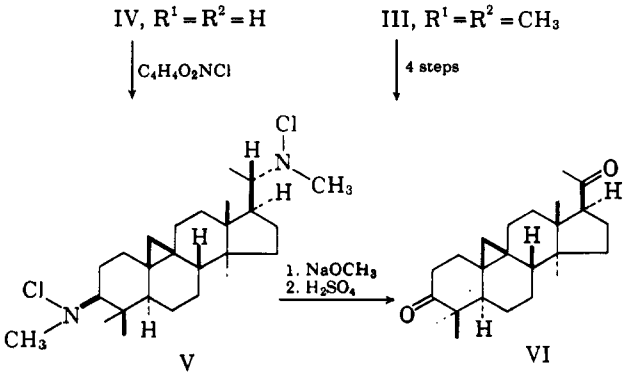
(12) D. Stauffacher, *Helv. Chim. Acta*, **47**, 968 (1964).

(13) K. S. Brown, Jr., and S. M. Kupchan, *J. Chromatog.*, **9**, 71 (1962).

(14) E. Schlittler, K. Heusler, and W. Friedrich, *Helv. Chim. Acta*, **32**, 2209 (1949).

(15) E. Schlittler and W. Friedrich, *ibid.*, **33**, 878 (1950).

interrelation of the alkaloid with cyclovirobuxine-D and with cycloartenyl acetate. Ruschig degradation of cycloprotobuxine-D proceeded, *via* the crystalline dichloramine V, $C_{26}H_{44}N_2Cl_2$, m.p. 164–165°, to the diketone VI (4,4,14α-trimethyl-9β,19-cyclo-5α-pregnane-3,20-dione), previously obtained from cyclovirobuxine-D (III, $R^1 = R^2 = CH_3$)[9] and from cycloartenyl acetate.[16] Configurations at C-3 and C-20 were assigned on the basis of biogenetic analogy to the companion alkaloids, cyclobuxine-D (I), cyclovirobuxine-D (III, $R^1 = R^2 = CH_3$), and cyclobuxamine-H (III, $R^1 = R^2 = H$).



Methylation of cycloprotobuxine-D with formaldehyde and formic acid gave dimethylcycloprotobuxine-D (IV, $R^1 = R^2 = CH_3$), and the latter compound was found to be identical with monomethylalkaloid L.[15] Calame and Arigoni have independently assigned the cycloprotobuxine-C structure (IV, $R^1 = H$, $R^2 = CH_3$) to alkaloid L.[10,16] The interrelation of cycloprotobuxine-D and cycloprotobuxine-C by conversion to the same methylation product[17] is in accord with expectation based on the structures assigned to the respective alkaloids.

Experimental[18,19]

Separation of the Acetone-Soluble Strong Bases by Partition Chromatography.—The acetone-soluble strong base fraction (2.5 g.)[3b] was dissolved in the upper phase of the system hexane–ethylene chloride–methanol–water (50:5:15:1) and chromatographed on a column of Celite 545 impregnated with phenol red and lower phase of the solvent system.[13] Four red bands were visible on the column, at R_f 0.95 (alkaloid L[14,15] = cycloprotobuxine-C[10]), 0.72 (cycloprotobuxine-D), 0.52 (buxenine-G[1]), and 0.50 (cyclobuxine-D[3]). The R_f 0.72 band was eluted, and the solution was evaporated to dryness under reduced pressure. The residue was dissolved in chloroform and extracted with 0.5 *N* hydrochloric acid solution. The acid solution was made alkaline with ammonium hydroxide and extracted with chloroform, and the chloroform extract was evaporated to dryness. Rechromatography of the crude product using the same partition system gave a yellow semisolid material (200 mg.), R_f 0.72, which was crystallized from acetone (100 mg.). Recrystallization from acetone yielded colorless needles (80 mg.), m.p. 140–142°, $[\alpha]^{28}D$ +112° (*c* 0.94, chloroform).

Anal. Calcd. for $C_{28}H_{46}N_2$: C, 80.76; H, 11.99; N, 7.25. Found: C, 80.78; H, 12.05; N, 7.29.

N,N′-Diacetylcycloprotobuxine-D (IV, $R^1 = R^2 = COCH_3$).—A solution of cycloprotobuxine-D (25 mg.) in dry pyridine (1 ml.) and acetic anhydride (0.5 ml.) was allowed to stand at room temperature for 18 hr. After evaporation to dryness under reduced pressure the residue was crystallized from acetone to yield colorless needles (20 mg.), m.p. 276–278°. The infrared spectrum showed a very strong band at 6.18 μ.

Anal. Calcd. for $C_{30}H_{50}N_2O_2$: C, 76.54; H, 10.71; N, 6.80. Found: C, 76.60; H, 10.64; N, 6.43.

N,N′-Dichlorocycloprotobuxine-D (V).—A solution of cycloprotobuxine-D (250 mg.) in chloroform (10 ml.) was cooled to 0° and treated dropwise with stirring with a solution of N-chlorosuccinimide (180 mg.) in chloroform (5 ml.). After stirring for 10 min. at 0° the solution was washed with water (three 20-ml. portions) and evaporated to dryness under reduced pressure. Crystallization of the residue from acetone gave colorless plates (277 mg.), m.p. 164–165°.

Anal. Calcd. for $C_{26}H_{44}Cl_2N_2$: C, 68.60; H, 9.67; N, 6.15. Found: C, 68.77; H, 9.83; N, 6.04.

Ruschig Degradation of N,N-Dichlorocycloprotobuxine-D.—The dichloramine V (100 mg.) was treated with a solution of sodium (0.5 g.) in methanol (20 ml.), and the mixture was heated under reflux for 2 hr. After evaporation to dryness under reduced pressure, the residue was treated with 0.5 *N* hydrochloric acid and chloroform. The chloroform extract was evaporated to dryness, the residue was dissolved in ethanol (10 ml.) and 6 *N* sulfuric acid (5 ml.), and the solution was allowed to stand at room temperature for 12 hr. The mixture was diluted with water and extracted with chloroform, and the chloroform extract was evaporated to dryness. The residue was chromatographed on Woelm neutral alumina (5 g.) using benzene (50 ml.) and 5% ethyl acetate in benzene (30 ml.) as eluents. The latter solvent mixture yielded a residue which was crystallized from acetone to yield colorless needles (29 mg.), m.p. 193–196°. The infrared spectrum was superimposable upon that of an authentic sample of 4,4,14α-trimethyl-9β,19-cyclo-5α-pregnane-3,20-dione (VI),[9] and the melting point was not depressed upon admixture with the authentic sample.

N,N-Dimethylcycloprotobuxine-D (IV, $R^1 = R^2 = CH_3$).—A solution of cycloprotobuxine-D (15 mg.) in 40% formaldehyde (15 mg.) and 88% formic acid (30 mg.) was heated under reflux for 12 hr. The reaction mixture was poured into 0.5 *N* hydrochloric acid solution; the acid solution was washed with ether, made alkaline with ammonium hydroxide, and extracted with chloroform. Evaporation to dryness gave a residue which was crystallized from chloroform–acetone to yield colorless plates (12 mg.), m.p. 208–211°. The melting point was not depressed upon admixture of a sample (m.p. 209–211°) of monomethylalkaloid L,[15] and the infrared spectra of the respective samples were identical.

Anal. Calcd. for $C_{28}H_{50}N_2$: C, 81.09; H, 12.15; N, 6.76. Found: C, 81.24; H, 12.27; N, 6.91.

(16) J. P. Calame and D. Arigoni, *Helv. Chim. Acta.*, in press. We thank Professor Arigoni cordially for informing us of these results prior to publication.

(17) Dr. R. Goutarel has kindly informed us of his isolation from *Buxus balearica* Willd. of cycloprotobuxine-A (= monomethylalkaloid L = dimethylcycloprotobuxine-D; IV, $R^1 = R^2 = CH_3$).

(18) Melting points are corrected to the nearest degree. Infrared spectra were measured in chloroform solution on a Beckman Model IR-5A spectrophotometer. Rotations have been approximated to the nearest degree. N.m.r. spectra were determined on a Varian A-60 spectrometer. Microanalyses were performed by Mr. Joseph Alicino (Metuchen, N. J.).

(19) We are grateful to Ciba Pharmaceutical Company for procurement and large-scale extraction of plant material, and especially thank Drs. E. Schlittler, D. Dickel, and K. Heusler for their kind interest and cooperation in this project.

Steroids. CCLXXV. The Aromatization of 10β-Acetoxyestr-4-ene-3,17-dione in the Presence of Amines*,[1]

FRANCISCO S. ALVAREZ[2] AND AUGUSTO B. RUIZ

The Research Laboratories, Syntex, S. A., Apartado 2679, Mexico, D.F., Mexico

Received November 16, 1964

In a recent publication[3] we described the facile aromatization of 10β-acetoxyestr-4-ene-3,17-dione and 10β-acetoxy-19-norpregn-4-ene-3,20-dione in alcoholic

* To Professor Louis F. Fieser.

(1) Steroids. CCLXXIV: A. D. Cross, E. Denot, R. Acevedo, and P. Crabbe, *Steroids*, submitted for publication.

(2) To whom correspondence should be addressed to the Syntex Research Center, Stanford Industrial Park, Palo Alto, Calif.

(3) F. S. Alvarez, *Steroids*, **3**, 13 (1964).