Notes

Rauwolfia Alkaloids. XLIX. Ring E Isomers in the Epiallo Series*

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Recent interest[2] in our report[3-5] of the isomerization of methyl reserpate to methyl neoreserpate prompts us to record some of our early unpublished observations.[6] The eight possible ring E epimers of the epiallo (hydrogens, 3β, 15α, 20α) series are shown in partial formulas Ia–VIIIa, and their probable chair–chair conformations are shown in partial formulas Ib–VIIIb. The steric effects which have been assumed to determine the specific chair–chair conformations Ib–VIIIb are, in decreasing order of priority, (1) the avoidance of an axial 16β-carbomethoxyl group, (2) the avoidance of an axially oriented C-2, and (3) the minimizing of the number of axial substituents on ring E.

Structure Ia is represented by methyl reserpate (R′ = H) and its many derivatives[7]; conformation Ib is consistent with the nuclear magnetic resonance[4b] and infrared[5] spectra. Epimer IIa (*e.g.*, R′ = H or CH_3) was prepared by Robison and co-workers[8] by alcoholysis of methyl reserpate *p*-bromobenzenesulfonate (I, R′ = $SO_2C_6H_4Br$); the infrared spectra of these derivatives showed no extra peaks in the 2700–2900 cm.$^{-1}$ region,[9] consistent[5] with conformation IIb and its equatorial 3β-hydrogen. Epimer IIIa (R′ = H or $COCH_3$) would be expected to show extra peaks in the 2700–2900-cm.$^{-1}$ region of the infrared, characteristic of axial C-3 hydrogens[5] and consistent with conformation IIIb. Epimer IVa (R′ = CH_3) has now been prepared by treating methyl 18-epireserpate methyl ether[8] (IIa, R′ = CH_3) with sodium methoxide in refluxing methanol. In this reaction, epimerization of the C-16 carbomethoxyl group of IIa (R′ = CH_3) gave the more stable isomer IVa (R′ = CH_3) which had only one minor element of instability (axial C-17 methoxyl group, IVb); this instability was apparently not sufficient to cause further epimerization of C-17 *via* methanol elimination–methanol addition.[3,4a] Compound IVa (R′ = CH_3) had infrared peaks at 2814 and 2760 cm.$^{-1}$ (chloroform), as predicted for the axial proton on C-3 of conformation IVb (R′ = CH_3). Compounds of partial formulas Va and VIa are unknown. Conformation VIb has large axial substituents at C-3, C-17, and C-18, while the alternate chair–chair conformation would have an axial C-16β carbomethoxyl group as a major element of instability; possibly the boat form (or twist boat) for ring E would be most stable for VIa. Epimer VII is represented by methyl neoreserpate[3] and its derivatives[10,11]; the infrared spectra of these compounds have peaks in the 2700–2900-cm.$^{-1}$ region characteristic of the axial C-3 hydrogen.[5] Epimer VIIIa (R′ = CH_3) was prepared by methanolysis of methyl neoreserpate *p*-bromobenzenesulfonate (VIIa, R′ = $SO_2C_6H_4Br$), following the C-18 epimerization method of Robison, *et al.*[8] The infrared spectrum of VIIIa (R′ = CH_3) was consistent with the presence of an axial C-3 hydrogen (conformation VIIIb).

The epimerization reactions VII (R′ = $SO_2C_6H_4Br$) → VIII (R′ = CH_3) and II (R′ = CH_3) → IV (R′ = CH_3) were accompanied by side reactions. One side product isolated from the methanolysis of VII (R′ = $SO_2C_6H_4Br$) was a *p*-bromobenzenesulfonate salt of a $C_{23}H_{28}N_2O_4$ compound, different from but isomeric with the Δ^{16}-enol ether product formed from elimination of *p*-toluenesulfonic acid from methyl neoreserpate tosylate.[3] Brief treatment of this new $C_{23}H_{28}N_2O_4$ salt with methanolic hydrogen chloride converted it to the hydrochloride salt of what is presumably a 17-keto 16-ester. In the epimerization of II (R′ = CH_3) to IV (R′ = CH_3) with sodium methoxide in methanol, a side product which was present in small amounts after a few hours of

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(6) The original investigation was intended to extend the work reported in previous papers.[3-5] In particular, the goals were to examine the scope of the sodium methoxide–methanol epimerization reaction, to prepare ring E epimers of the epiallo series, and to test predictions concerning ring E isomers based on considerations of predicted conformations. This note describes the preparation of two new epimers, bringing the total number reported to five (of the eight which are possible). The work reported here was completed in 1961 before the investigation was discontinued.

(7) For leading references see (a) E. Schlittler in "Rauwolfia: Botany Pharmacognosy, Chemistry, and Pharmacology," R. E. Woodson, Jr., H. W. Youngken, E. Schlittler, and J. A. Schneider, Ed., Little, Brown and Co., Boston, Mass., 1957, pp. 74–96; (b) P. E. Aldrich, P. A. Diassi, D. F. Dickel, C. M. Dylion, P. D. Hance, C. F. Huebner, B. Korzun, M. E. Kuehne, L. H. Liu, H. B. MacPhillamy, E. W. Robb, D. K. Roychaudhuri, E. Schlittler, A. F. St. André, E. E. van Tamelen, F. L. Weisenborn, E. Wenkert, and O. Wintersteiner, *J. Am. Chem. Soc.*, **81**, 2481 (1959).

(8) M. M. Robison, R. A. Lucas, H. B. MacPhillamy, R. L. Dziemian, I. Hsu, and R. J. Kiesel, *ibid.*, **83**, 2694 (1961).

(9) We thank Drs. Robison, Lucas, and MacPhillamy for making these infrared curves available for our inspection.

(10) Methyl neoreserpate was prepared[3] by refluxing a solution of methyl reserpate in methanol, in the presence of sodium methoxide, for 64 hr. The 74.4% by weight of methylene chloride soluble product was composed of 47% crude methyl neoreserpate and approximately 24% methyl reserpate.[4a] A further 10% of the methylene chloride soluble product has now been found to be methyl neoreserpate N-oxide, m.p. 224–225°, $[\alpha]_D$ −74.5° ($CHCl_3$).

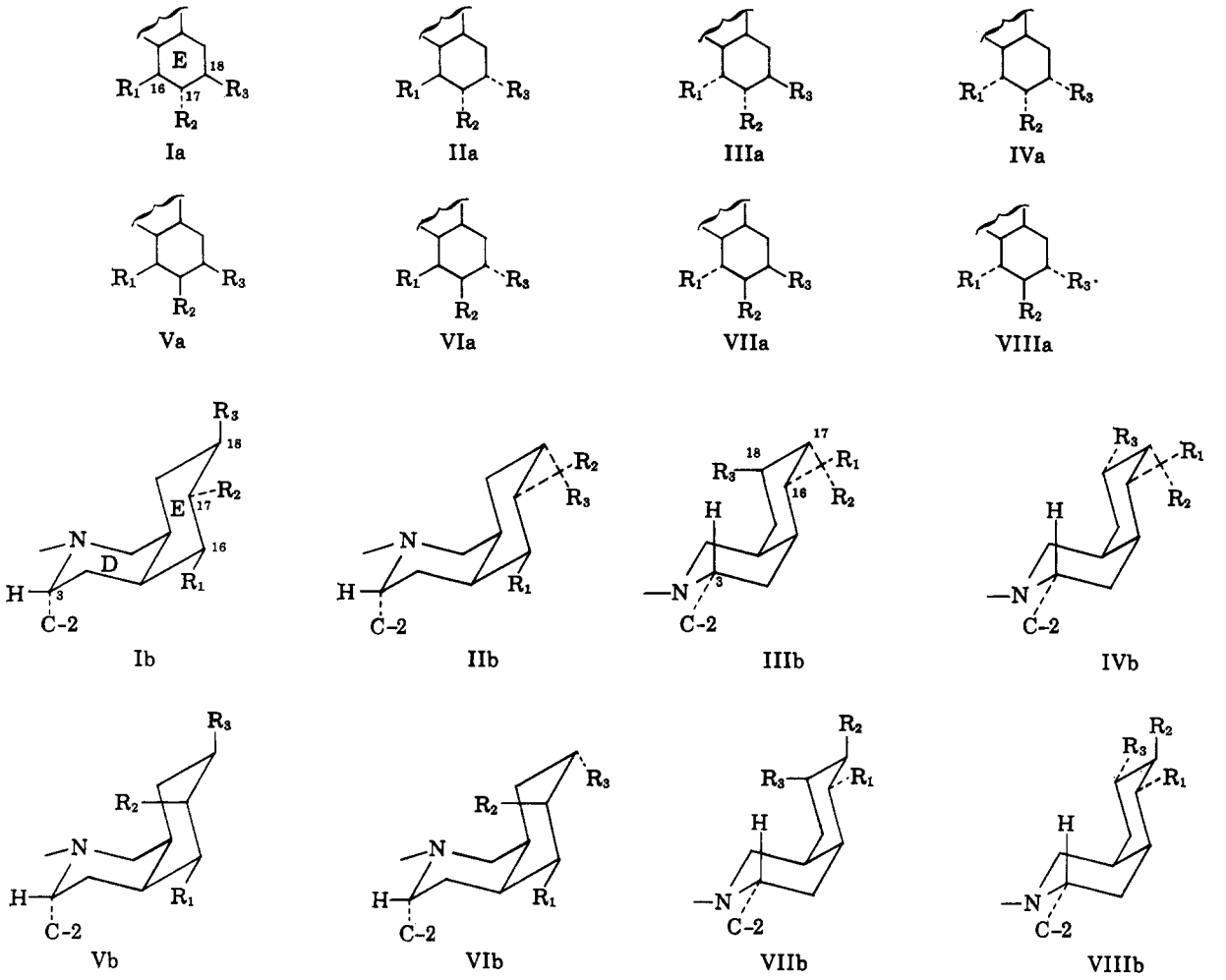
Anal. Calcd. for $C_{23}H_{30}N_2O_6 \cdot 0.5H_2O$ (439.52): C, 62.85; H, 7.11; N, 6.37. Found: C, 63.19; H, 7.36; N, 6.47.

(11) Methyl neoreserpate was treated with diazomethane and fluoroboric acid in methylene chloride at −10°, in a manner similar to that described by Robison, *et al.*,[8] to give methyl neoreserpate methyl ether methofluoroborate in 50% yield. One crystallization from methanol gave white prisms, m.p. 318–321°, $[\alpha]_D$ −41.3° (pyridine).

Anal. Calcd. for $C_{25}H_{35}BF_4N_2O_5$ (530.39): C, 56.61; H, 6.65; N, 5.28. Found: C, 56.39; H, 6.68; N, 5.21.

The methofluoroborate salt was converted to methyl neoreserpate methyl ether methochloride by passage through a column of Amberlite IRA-400 ion-exchange resin (chloride form), as described by Robison, *et al.*[8] The pure methochloride salt had m.p. 277–279°, $[\alpha]_D$ +5.1° ($CHCl_3$).

Anal. Calcd. for $C_{25}H_{35}ClN_2O_5$ (479.03): C, 62.68; H, 7.36; N, 5.85. Found: C, 62.49; H, 7.47; N, 5.64.



R_1 = $-COOCH_3$, R_2 = $-OCH_3$, R_3 = $-OR'$

refluxing became the major product after several days of refluxing. When isolated, it proved to be yet another $C_{23}H_{28}N_2O_4$ isomer, resulting from the elimination of methanol; its infrared spectrum showed bands characteristic of a 3-axial hydrogen[5] (probably 3β-hydrogen and 16α-carbomethoxyl) and of an enol ether (possibly Δ^{17}-enol 18-ether).

Experimental[12]

Preparation of VIII (R' = CH_3).—A suspension of 1.94 g. of methyl neoreserpate *p*-bromobenzenesulfonate (VII, R' = $SO_2C_6H_4Br$)[3] in 147 ml. of anhydrous methanol containing 0.37 g. of triethylamine was stirred at 95–100° in a pressure bottle for 22 hr. The turbid yellow solution was stripped to dryness at reduced pressure, and the 2.21 g. of brown residue was taken up in 50 ml. of methylene chloride, washed with aqueous sodium bicarbonate and saturated sodium chloride solution, and dried over anhydrous sodium sulfate. The filtered solution was stripped to dryness at reduced pressure, and the 1.77 g. of brown semisolid residue was triturated with ethyl ether, changing to a tan granular solid. The solid was collected and dried, giving 1.22 g., m.p. 195–197°. A mixture of 183 ml. of benzene and 63 ml. of cyclohexane was boiled with the solid, leaving 0.13 g. of starting material as an insoluble tan solid, and the solution was passed through a column of decolorizing charcoal, concentrated to a volume of 12 ml., and diluted with 12 ml. of warm cyclohexane. After removal of 0.24 more g. of starting material, the solution was twice concentrated to a volume of 12 ml. and diluted with 12 ml. of cyclohexane. Chilling at 5° afforded 0.43 g. (32.8%) of a buff-colored solid, m.p. 205–211°, which was crystallized from methanol to give light yellow crystals of VIII (R' = CH_3): m.p. 223–225°; $[\alpha]D$ +34.7°; ν_{max}^{Nujol} (cm.$^{-1}$) 3423, 2857, 2740, 1739, 1631, 1160, and 1076; $\nu_{max}^{CHCl_3}$ (cm.$^{-1}$) 3468, 3356, 2930, 2835, 2770 (sh), 1733, 1631, 1273, and 1152.

Anal. Calcd. for $C_{24}H_{32}N_2O_5$ (428.54): C, 67.27; H, 7.53; N, 6.54. Found: C, 67.22; H, 7.57; N, 6.61.

In another run, a suspension of 3.00 g. of VII (R' = $SO_2C_6H_4Br$) in 160 ml. of methanol containing 0.57 g. of triethylamine was magnetically stirred and heated at 95–100° for 72 hr. After standing at room temperature, 1.50 g. (50%) of white crystalline starting material precipitated and was removed. The solvent was removed at reduced pressure and the 1.71 g. of yellow-brown glassy residue was slurried with 60 ml. of benzene, leaving 0.57 g. (19%) of insoluble yellow solid, m.p. 185–215°. The solid was taken up in 10 ml. of methanol, the solution was decolorized by passing through a layer of activated charcoal, and the light yellow solution was concentrated and chilled, giving 0.26 g. of off-white crystals: m.p. 232–233°; $[\alpha]D$ +17° (pyridine); ν_{max}^{Nujol} (cm.$^{-1}$) 3475 (sh), 3321, 2707, 2621, 1742, 1639, 1240, and 1178.

Anal. Calcd. for $C_{29}H_{33}BrN_2O_7S\cdot H_2O$ (651.60): C, 53.46; H, 5.41; N, 4.30. Found: C, 53.66; H, 5.63; N, 4.44.

A solution of 0.20 g. of these off-white crystals in 1.0 ml. of hot methanolic hydrogen chloride was diluted with 20 ml. of acetone and chilled. The solid was collected, washed with acetone, and dried, giving 0.10 g. (76%) of white crystals: m.p. 261–263°; $[\alpha]D$ +32.9 (MeOH–H_2O); ν_{max}^{Nujol} (cm.$^{-1}$) 3542 (sh), 3341 (sh), 3180, 2680, 2557, 1750, 1727, 1638, 1275, and 1168.

Anal. Calcd. for $C_{22}H_{26}N_2O_4\cdot HCl$ (418.93): C, 63.08; H, 6.50; N, 6.69. Found: C, 62.92; H, 6.80; N, 6.20.

Preparation of IV (R' = CH_3).—A suspension of 10.00 g. of methyl 18-epireserpate methyl ether (II, R' = CH_3) hydrochloride[3] in 140 ml. of anhydrous methanol containing 2.8 g. of sodium methoxide was stirred and refluxed for 48 hr. Dilution with

(12) Optical rotations were taken in chloroform solution at 24–28° unless otherwise specified. Melting points were determined in an electrically heated aluminum block and are uncorrected. The alkaloids either darkened or decomposed at their melting points. Analytical samples were routinely dried under vacuum at 75° for 3–5 hr. Paper chromatography was used in all cases to establish homogeneity and to confirm identity or nonidentity of materials.

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]

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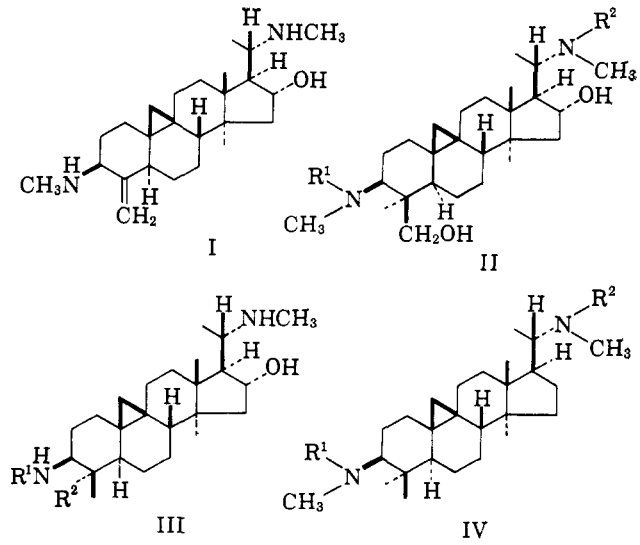
In 1962 we reported the elucidation of the structure[3] and configuration[4] of cyclobuxine-D (I), an alkaloid isolated from *Buxus sempervirens* L.[5] Cyclobuxine-D was shown to be the prototype of a new class of steroidal alkaloids which contains a cyclopropane ring and which has a substitution pattern 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

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(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).

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