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(11) For these experiments, because of difficulties in isolating the product from the solvent in the peracetic acid epoxidation of **1**, pure **2** was best prepared according to E. J. Reist, I. G. Junga, and B. R. Baker, *J. Org. Chem.*, **25**, 1673 (1960); the only modification being that the dehydrobromination was performed by treatment of the distilled isoprene bromohydrin (bp 60 °C, 10 mm) with solid sodium hydroxide at 90 °C and atmospheric pressure in a distillation flask equiped with a Vigreux fractionating column. Sodium sulfate drying of the distillate (bp 72–73 °C) afforded pure **2**.
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(14) Lithium chloride is a catalyst of oxidative halogenations of carbonyl compounds with cupric chloride; see literature cited under ref 12.
(15) For the acetoxylation of **3** to **4**, a procedure (see Experimental Section) different from the one cited under ref 2 gave more satisfactory results.
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Hydroxyl Assisted Epoxide Opening in Picrotoxins

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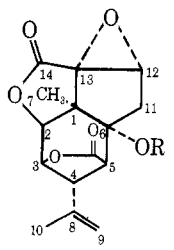
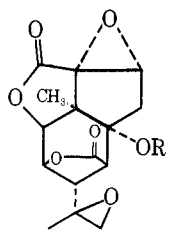
Received December 9, 1975

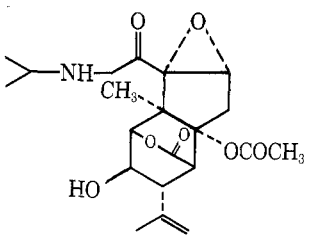
It is well documented that picrotoxinin (**1a**) is the potent analeptic component of picrotoxin.[1] In order to modify the analeptic properties of **1a**, we were interested in the introduction of a basic nitrogen moiety in the picrotoxinin molecule. Picrotoxinin possesses an epoxide ring which should theoretically be opened with amines or other nucleophiles, but is known to be very resistant to intermolecular nucleophilic attack. This unusual feature of the picrotoxinin structure is explained on the basis of shielding of the epoxide ring from rearward attack by the lactone groupings. However, the close proximity of the axial C-6 α-hydroxyl group to the C-4 α-isopropenyl group in **1** suggested to us that the 8,9-epoxide of picrotoxinin (i.e., **2a**) would be prone to nucleophilic attack due to participation by the C-6 hydroxyl group in oxirane ring opening. Indeed, this was found to be the case since epoxidation of **1a** with *m*-chloroperbenzoic acid in methylene chloride followed by aqueous work-up resulted in a mixture of the desired epoxide **2a** and the glycol **4d**. It is interesting to note that previous workers have reported similar problems in the synthesis of this epoxide.[2] In contrast, no difficulty was experienced in the epoxidation of picrotoxinin 6-acetate (**1b**) to give **2b** by the same procedure. Hence picrotoxinin epoxide (**2a**) appears to be much more reactive than **2b**. Because of this reactivity the epoxide **2a** was not isolated in pure form but was used, after a modified work-up procedure, directly in subsequent reactions with amines. On treatment of **2a** with pyrrolidine or diethylamine at room temperature, the corresponding amine derivatives **4a** and **4b** were obtained whereas chloroform reflux conditions were required to obtain **4c** from **2b** with diethylamine.



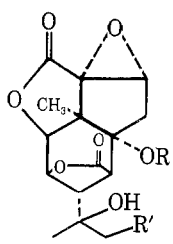
1a, R = H (picrotoxinin)
b, R = $COCH_3$



2a, R = H
b, R = $COCH_3$



3



4a, R = H; R′ = N⟨ ⟩
b, R = H; R′ = $N(C_2H_5)_2$
c, R = $COCH_3$; R′ = $N(C_2H_5)_2$
d, R = H; R′ = OH
e, R = H; R′ = H (picrotin)

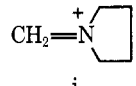
The ease of opening of the epoxide in **2a** with amines to yield compounds of type **4** can be ascribed to the neighboring 6-hydroxyl group participation in an "anionic" process. Only a few examples of such participation are known such as the neighboring hydroxy group participation in the alkaline hydrolysis of esters[3–5] and more recently the opening of epoxides by nucleophiles in steroids.[6] Neighboring group participation in "cationic" reactions, on the other hand, has been known for many years and is well understood.[3,7,8]

The structural assignment of these compounds (**4a–c**) seems secure on the basis of spectral data. The NMR spectra of **4a** and **4b** are compared in Table I with those of picrotoxinin (**1a**) and picrotin (**4e**). The NMR spectra of the latter two important compounds have apparently not been reported previously. Infrared spectra of the amines show that the lactone groupings are maintained in the picrotoxinin epoxide molecule during reaction with amines. The mass spectrum of **4a** showed principal ions at *m/e* 380 $(M + 1)^+$, 379 $(M^{\cdot+})$, 280, 128, and 84 (base). The base peak at *m/e* 84 and the peak at *m/e* 128 correspond to the ions i and ii, respectively, both of which confirm the presence and

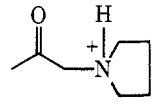
Table I. NMR Assignments of Picrotoxin Derivatives (δ, acetone-d_6)

Proton	Picrotoxinin (1a)	Picrotin (4e)	4a	4b
C_2	5.08, d $J_{2,3}$ = 4	4.88, d $J_{2,3}$ = 3.5	4.91, d $J_{2,3}$ = 3.5	4.90, d $J_{2,3}$ = 3.5
C_3	5.23, dd $J_{2,3}$ = 4; $J_{3,4}$ = 4	5.08, ddd $J_{2,3}$ = 3.5; $J_{3,4}$ = 4.0, $J_{3,5}$ = 1	5.11, dd $J_{2,3}$ = 3.5; $J_{3,4}$ = 5.0	5.12, dd $J_{2,3}$ = 3.5; $J_{3,4}$ = 4.5
C_4 C_5	3.6, b 3.0, d[a]	2.8–3.1, m	2.6–3.2, m[a]	2.8–3.1, m
C_7	1.21, s	1.22, s	1.23, s	1.23, s
C_9	4.98, bs	1.49, s	2.80, s	2.66
C_{10}	1.94, bs	1.46, s	1.46, s	1.43, s
C_{11}	α, 1.86, d[a] β, 2.88, dd $J_{11,11}$ = 15; $J_{11,12}$ = 3.5	α, 1.87, d J = 15 β, 2.82, dd; $J_{11,11}$ = 15; $J_{11,12}$ = 3.5	α, 1.98, d[a] $J \sim 15$ β, 2.82, dd[a] $J_{11,11} \sim 15$; $J_{11,12} \sim 3.5$	α, 2.02, d $J \sim 15$ β, 2.83, dd[a] $J_{11,11} \sim 15$; $J_{11,12} \sim 3.5$
C_{12}	3.60, d; $J_{11,12}$ = 3.5	3.58, d; $J_{11,12}$ = 3.5	3.58, d; $J_{11,12}$ = 3.5	3.60, d; $J_{11,12}$ = 3.5
OH	2.87, bs	5.52, 5.87, 2 bs	4.37, b	4.8, b
			1.6–1.9, m; 2.6–2.9, m	
				0.99, t 2.69, q } J = 7.0

[a] Multiplet partially obscured.

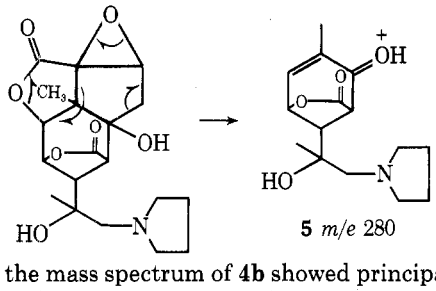


i



ii

establish the position of the pyrrolidine moiety in **4a**. The peak at *m/e* 280 is rationalized as arising from **5** as shown.



5 *m/e* 280

Similarly, the mass spectrum of **4b** showed principal ions at *m/e* 381 ($M^{\cdot+}$), 282, 130, and 86 (base) indicating a fragmentation pattern identical with that of **4a**, the increase in mass by 2 units being due to the substitution of pyrrolidine by diethylamine moiety.

A different course of reaction takes place on treatment of picrotoxinin acetate (**1b**) with isopropylamine at room temperature for 16 h, which results in an attack on the C-14 lactone with concomitant opening of the C-15 lactone to form the amide **3**, similar to the formation of hydroxy-α-picrotoxinic acid from 6-acetylpicrotin with dilute alkali.[9]

Experimental Section

Melting points are uncorrected and were determined on a Thomas-Hoover capillary melting point apparatus. NMR spectra were measured on a Varian T-60 spectrometer and ir spectra were determined on a Perkin-Elmer Model 700 instrument.

8,9-Epoxypicrotoxinin 6-Acetate (2b). To a solution of 2.34 g (0.007 mol) of picrotoxinin 6-acetate[10] in 50 ml of methylene chloride was added a solution of 1.21 g (0.007 mol) of *m*-chloroperbenzoic acid (85%) in 50 ml of methylene chloride and the mixture was refluxed for 24 h. After cooling, a 10% aqueous solution of sodium sulfite was added and the organic layer was separated. The aqueous layer was twice extracted with methylene chloride and the combined extracts were washed with bicarbonate solution followed with water and dried. After evaporation of the solvent in vacuo, a white solid was obtained which was crystallized from chloroform–methanol mixture to give colorless needles: mp 175–177 °C; NMR ($CDCl_3$) δ 1.43 (6 H, s, C_1 CH_3, C_8 CH_3), 2.08 (3 H, s, acetate), 2.10 (1 H, d, $J_{11,11}$ = 15.5 Hz, C_{11} Hα), 2.54, 2.78 (2 H, AB, J = 4.4 Hz, C_9 H_2), 3.30 (1 H, dd, $J_{4,5}$ = 4.5, $J_{3,5}$ = 5.0 Hz, C_4 H), 3.57 (1 H, dd, $J_{11,11}$ = 15.5, $J_{11,12}$ = 3.5 Hz, C_{11} Hβ), 3.72 (1 H, d, $J_{4,5}$ = 4.5 Hz, C_5H), 3.79 (1 H, d, $J_{11,12}$ = 3.5 Hz, C_{12} H), 4.63 (1 H, d, $J_{2,3}$ = 3.5 Hz, C_2 H), 5.02 (1 H, dd, $J_{2,3}$ = 3.5, $J_{3,4}$ = 5.0 Hz, C_3 H); ir (KBr) 1790 (lactone), 1735 (acetate), 860, 830 cm^{-1} (epoxide). Anal. Calcd for $C_{17}H_{18}O_8$: C, 58.25; H, 5.18. Found: C, 58.04; H, 5.32.

8,9-Epoxypicrotoxinin (2a). To a solution of 2.34 g (0.008 mol) of picrotoxinin in 50 ml of methylene chloride was added a solution of 1.38 g (0.008 mol) of *m*-chloroperbenzoic acid (85%) in 40 ml of methylene chloride and the mixture was refluxed for 27 h. After cooling, solid sodium sulfite was added to the mixture and stirred for 0.5 h. The solution was filtered and evaporated to leave a white solid: mp 100–105 °C; ir (KBr) 3450 (OH), 1800, 1770 (lactones), 860, 830 cm^{-1} (epoxide). Without further purification, this material was used in subsequent reactions with amines. When the epoxide **2a** was worked up as **2b** (aqueous work-up) a considerable amount of glycol **4d** was formed[2] (positive periodate test).

***N*-Isopropylpicrotoxininamide 6-Acetate (3).** A mixture of 0.33 g (0.001 mol) of picrotoxinin 6-acetate (**1b**) and 3 ml of isopropylamine was stirred at room temperature. After 16 h, the product was evaporated in vacuo and the residue was purified by preparative TLC (2-mm thick silica gel, Merck, ether) to yield a gum: NMR ($CDCl_3$) δ 1.13 [d, J = 6.5, Hz, $CH(CH_3)_2$], 1.37 (s, C_1 CH_3), 1.79 (bs, C_8 CH_3), 1.90 (s, acetate), 2.3–2.7 (bm, C_{11} H_2), 3.77 s, 3.95 d, J = 2 Hz, 3.7–4.3 m [C_5 H, C_{12} H, C_4 H, $CH(CH_3)_2$], 4.53 (bs, exchangeable, OH), 4.59 (d, J = 5.5 Hz, C_3 H), 5.00 (b=CH_2), 5.52 (s, C_2 H), 6.33 (d, J = 8 Hz, exchangeable, NH); ir ($CHCl_3$) 3430 (OH), 1760 (δ-lactone), 1740 (acetate), 1665 (amide), 1520, 1380, 1280, 1065, 1055 cm^{-1}. Anal. Calcd for $C_{20}H_{27}NO_7$: C, 61.05; H, 6.92; N. 3.56. Found: C, 61.11; H, 6.79; N, 3.51.

8-Hydroxy-9-pyrrolidinopicrotoxinin (4a). To 0.462 g (0.0015 mol) of 8,9-epoxypicrotoxinin, an excess of pyrrolidine was added and the orange-colored solution was stirred at room temperature for 1 h. The pyrrolidine was removed in vacuo and 5 ml of 4 N hydrochloric acid was added. After filtration the solution was extracted with ether. The acid solution was neutralized with sodium bicarbonate and extracted with chloroform. The combined chloroform extracts were washed, dried, and evaporated to leave a solid. It was purified by preparative TLC (2-mm thick silica gel, Merck, 20% methanol–chloroform) followed by crystallization from chloroform–ether to yield colorless crystals: mp 149–151 °C; NMR (see Table I); mass spectrum *m/e* (rel intensity) 380 (0.21), 379 (0.18), 364 (0.95), 280 (6.86), 128 (3.63), 84 (100); ir (KBr) 3400 (OH), 1790 (five-membered lactone), 1140, 980 cm^{-1}. Anal Calcd for $C_{19}H_{25}NO_7$: C, 60.15; H, 6.64; N, 3.69. Found: C, 60.09, H, 6.65; N, 3.58.

8-Hydroxy-9-diethylaminopicrotoxinin (4b). To 0.616 g (0.002 mol) of 8,9-epoxypicrotoxinin, an excess of diethylamine was added and the orange solution was stirred at room temperature for 0.5 h. After evaporation in vacuo and similar work-up as in **4a**, a solid was obtained which was purified by preparative TLC (2-mm thick silica gel, Merck, 20% methanol–chloroform): mp 78–81 °C; NMR (see Table I); mass spectrum *m/e* (rel intensity) 382 (0.62), 381 (0.45), 366 (1.82), 282 (18.0), 130 (6.37), 86 (100), ir ($CHCl_3$) 3400 (OH), 1800 (γ-lactone), 1300, 1260, 1140, 1000 cm^{-1}.

8-Hydroxy-9-diethylaminopicrotoxinin 6-Acetate (4c). To a solution of 0.35 g (0.001 mol) of **2b** in 12 ml of chloroform containing a catalytic amount of *p*-toluenesulfonic acid was added 4 ml of diethylamine. After refluxing for 3 h, the solvent was removed and dilute hydrochloric acid was added. The mixture was extracted with ethyl acetate and the acid solution was evaporated in vacuo to leave a gummy solid: NMR ($CDCl_3$) δ 0.98 (t, J = 7.0 Hz, NCH_2CH_3), 1.33 (s, C_1 CH_3), 1.40 (s, C_8 CH_3), 2.10 (s, acetate), 2.09 (d, $J_{11,11}$ = 15 Hz, C_{11} Hα), 2.40 (s, C_9H_2), 2.65 (q, J = 7.0 Hz, NCH_2CH_3), 3.3 (m, C_4 H), 3.59 (dd, $J_{11,11}$ = 15, $J_{11,12}$ = 3.5 Hz, C_{11} Hβ), 3.73–3.87 (m, C_5 H, C_8 H), 4.93–5.13 (m, C_2 H, C_3H); ir (KBr) 3450 (OH), 1790 (γ-lactone), 1730 (acetate), 1260, 1160, 1000 cm^{-1}. Anal. Calcd for $C_{21}H_{29}NO_8$·HCl: C, 54.84; H, 6.59; N, 3.04, Cl, 7.71. Found: C, 55.27; H, 6.90; N, 3.17; Cl, 7.80.

Acknowledgment. This work was carried out with the support of Office of Naval Research Contract N0014-71-C-0422. We are grateful to Professor John C. Sheehan for helpful discussions.

Registry No.—**1a,** 17617-45-7; **1b,** 17617-62-8; **2a,** 58281-91-7; **2b,** 58281-92-8; **3,** 58281-93-9; **4a,** 58281-94-0; **4b,** 58281-95-1; **4c** HCl, 58281-96-2; **4e,** 21416-53-5; isopropylamine, 75-31-0; pyrrolidine, 123-75-1; diethylamine, 109-89-7.

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Conformational Analysis of the 17(20) Bond of 20-Keto Steroids

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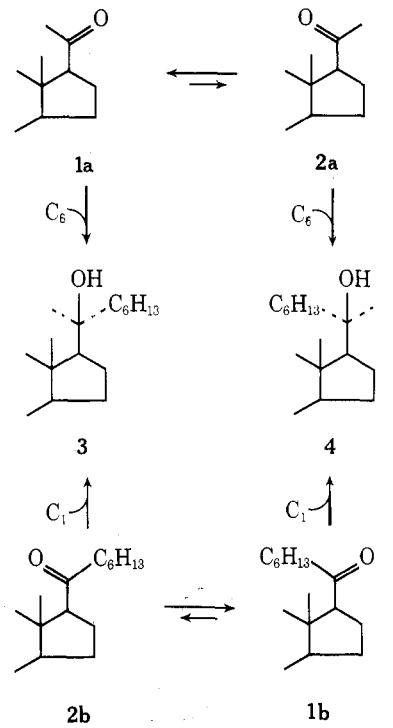
Received September 12, 1975

From the reduction of pregnenolone (**1a** and **2a**) and other 20-keto steroids as well as from other data, it has been concluded[1] that rotation about the 17(20) bond can occur and that the preferred conformation at the time of reaction is dependent on certain structural factors. Lithium aluminum hydride reduction of pregnenolone, for instance, yields a 2:1 mixture of the 20-hydroxy epimers.[2,3] As predicted qualitatively by Cram's rule, the dominant epimer is the one derived by α-side attack of the reagent on conformer **1a** in which C-21 is cis oriented relative to C-13. Surprisingly, however, the Grignard reaction of the same ketone is reported[4-6] to be completely stereospecific, yielding only the product from conformer **1a,** which led us to a reinvestigation of this problem.

Since the angular methyl group on C-13 should direct attack of the large Grignard reagent to the α face of the steroid, the product composition from the reaction of pregnenolone acetate (**1a** and **2a**) should indicate what the conformational equilibrium is about the 17(20) bond in the ketonic starting material. The α-hydroxy product (**3**) must be derived from the cis conformer (**1a**) with the methyl group



toward C-13 and the β-hydroxy product (**4**) from the trans conformer (**2a**) with the methyl group away from C-13. We have found that both epimers are indeed formed which implies that an equilibrium does exist between both conformers as expected from the work on reductions. Our data also show that conformer **1a** is present in the greater amount at the moment of reaction, since the α epimer (**3**) was present in greater amount in the product mixture, and this is in agreement with deductions based on dipole moment measurements[7] and application of the axial halo ketone rule.[8]

While the cis conformer (**1a**) will yield the 20α epimer (**3** from **1a**) when the incoming group is C_6, the 20β epimer (**4** from **1b**) should be formed when the incoming group is C_1. Furthermore, examination of molecular models reveals that the trans conformer should be destabilized when the alkyl group is increased in size owing to interaction of C-22 with C-16, and the ratio of cis to trans conformer should increase, i.e., the ratio of **1b** to **2b** should be greater than that of **1a** to **2a.** These conclusions require that 20-keto-21-norcholesteryl acetate, which is tantamount to 21-isovalerylpregnenolone acetate, should exist with conformer **1b** being present in larger amount compared to conformer **2b** than is so for conformer **1a** compared to **2a** in the pregnenolone case. The observed facts from the respective Grignard reactions are in agreement, since we find that the 20β epimer is formed in much greater amount from 21-isovalerylpregnenolone than is the 20α epimer from pregnenolone. The ratio of 20β to 20α epimer in the former case is 10:1.0 while the inverse ratio in the latter case is only 1.7:1.0.

In the NMR spectra of the 20α- and 20β-hydroxycholesterols (**3** and **4,** respectively) the signals for C-18 are exactly the same. The downfield chemical shift compared to cholesterol is δ 0.19 which agrees with expectation[9] for a 1,3-diaxial relationship between the 20-hydroxy group and C-18. Such a relationship coincides with the conformations of each of the epimers which would result from Grignard reagent attack on the ketone conformers (**3** from **1a** or **2b** and **4** from **1b** or **2a**). The spectrum thus implies conformational preference leading to the structure of the 20-hydroxycholesterol being "frozen" in the conformation existing at the end of the reaction. The 20α-hydroxy epimer (**3**)