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Synthesis of Analogues of GABA. X* The Bicyclic β-Diketone Octahydro-1*H*-2-pyrindine-5,7-dione

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Abstract

The synthesis is described of octahydro-1*H*-2-pyrindine-5,7-dione (4) and its 6-diazo and 6-bromo derivatives. These are analogues of GABA in which the carboxyl group is replaced by an enolic β-diketone. The compounds showed negligible or very weak activity as GABA agonists with respect to inhibition of [^{3}H]GABA binding, uptake and transamination in rat brain membranes.

Compounds such as nipecotic acid[1] (1) with an acidic functionality at the 3-position of a piperidine ring[2–4] selectively inhibit the cellular uptake of the inhibitory neurotransmitter γ-aminobutyric acid (GABA).[5] Others with an acidic functionality at the 4-position of the ring, such as isonipecotic acid (2),[3] piperidine-4-sulfonic acid[6] and the isoxazole analogue THIP (4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol) (3)[7,8] act at postsynaptic GABA receptors with minimal interaction at GABA uptake sites. β-Diketones, as their enolic tautomers, can be considered as vinylogues of carboxylic acids with comparable acidity.[9] Thus octahydro-1*H*-2-pyrindine-5,7-dione (4) may exist as tautomers (4a) and (4b) and is therefore capable of bearing an acidic functional group in positions corresponding to those of the acidic group in either (1) or (2). So the synthesis of (4) is of interest to ascertain whether such an analogue would interact with GABA uptake or receptor binding.

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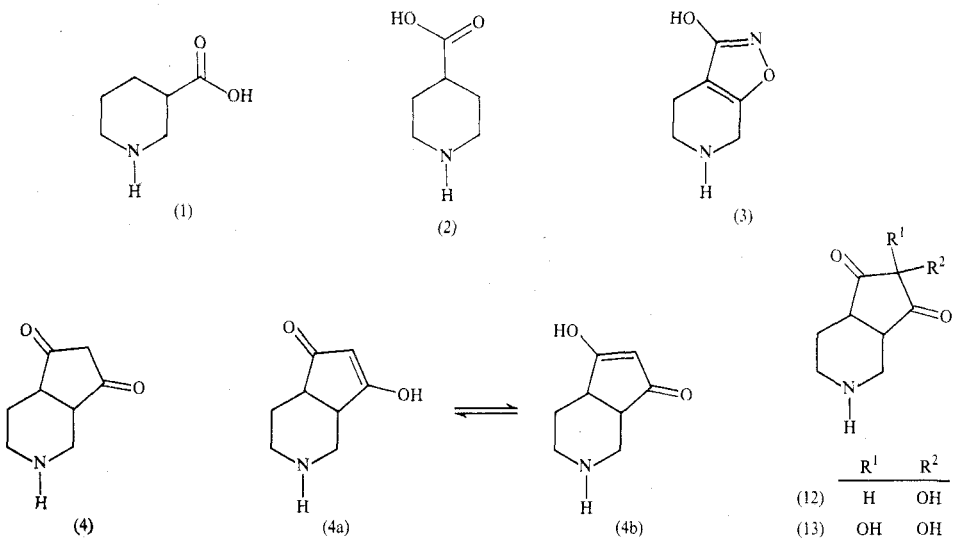
A common route to five-membered ring β-diketones has been the reaction of active methylene reagents with cyclic anhydrides.[10,11] Recently, Massy-Westropp *et al.* have shown that intermediates from Wittig reagents and unsaturated cyclic anhydrides readily form cyclopentene-1,3-diones, but intermediates from saturated cyclic anhydrides do not recyclize under similar basic conditions.[12] Consistent with this report, our preliminary attempts to form the cyclopentanedione ring of (4) via the saturated diacid (5) were unsuccessful.



We now report a synthesis of the saturated β-diketone (4) in which the cyclopentanedione ring was formed before saturation of the heterocyclic ring, as shown in Scheme 1. As previously reported[13,14] the anhydride from cinchomeronic acid condensed with ethyl acetoacetate and cyclized under basic conditions to give the intermediate pyridine diketo ester (6) (Scheme 1). The success of the route depended on selective reduction of the heterocyclic ring, and this was achieved by protecting the diketone as the enolic sodium salt during catalytic hydrogenation. Without isolation of the intermediates, acid hydrolysis and decarboxylation gave the desired product (4) after desalting over ion-exchange resin. If the hydrogenation step was carried out under neutral or acidic conditions, complex or unstable products were obtained. The ring junction in (4) was shown to have *cis* stereochemistry since oxidative cleavage[15] by use of excess sodium periodate gave the known *cis*-piperidine-3,4-dicarboxylic acid, characterized as the hydrochloride.

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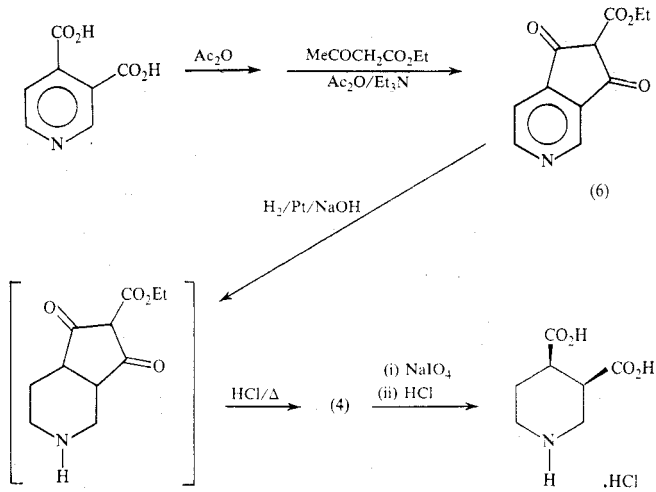
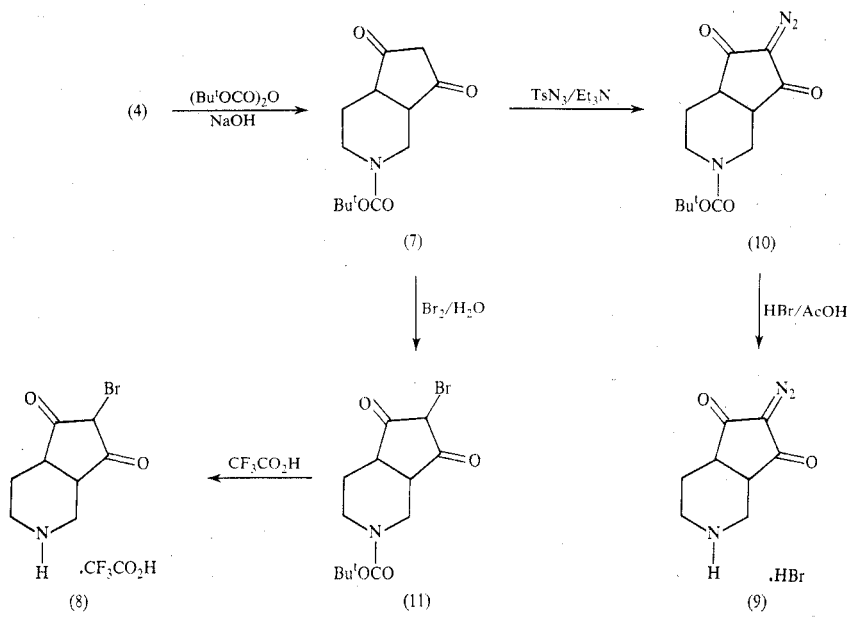
For chemical manipulation to get further GABA analogues, the nitrogen was protected with the t-butyloxycarbonyl group to yield (7) (Scheme 2). The bromo compound (8) and the diazo derivative (9) were prepared under the standard bromination and diazo transfer[16,17] conditions as shown in Scheme 2. The diazo hydrobromide salt (9), formed by hydrobromic acid/acetic acid deprotection of (10), was



Scheme 1



Scheme 2

[16] Oda, M., Kasai, M., and Kitahara, Y., *Chem. Lett.*, 1977, 307.
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considerably more stable than expected and could be recrystallized, in contrast to the trifluoroacetate salt (8) which decomposed on attempted purification. Hence it was characterized spectroscopically and the extent of bromination proved by full characterization of the nitrogen protected precursor (11).

Our attempts to isolate the oxidized derivatives (12) and (13) were unsuccessful. Tried routes to (12) included the displacement of the bromo substituent of (11) with potassium acetate, reaction of the sodium salt of (7) with dibenzoyl peroxide,[18] and 2 M hydrochloric acid hydrolysis of (9) which after 24 h reflux was substantially unchanged. Unsuccessful routes to (13) included oxidation of (10) with t-butyl hypochlorite,[19–21] and the tetrabutylammonium-fluoride-catalysed photooxidation of (7).[22] Our failure to isolate these oxidized products is consistent with the reported low stability of cyclopentane-1,2,3-trione systems even without the presence of a nitrogen functionality in the molecule.[23]

The bicyclic β-diketones (4), (8) and (9) were examined as GABA mimetic agents in rat brain tissues and were tested as inhibitors of the cellular uptake,[24] the enzymic transamination,[25] as well as the sodium-independent binding to rat brain membranes of radiolabelled GABA.[26] These compounds did not act as uptake inhibitors at 5×10^{-4} M nor did they significantly inhibit [^{3}H]GABA binding at 100 nM. Although compounds (4) and (8) were inactive at inhibiting transamination of GABA at 10^{-3} M, the diazo derivative (9) was weakly active and the inhibitor concentration that results in reduction of the transamination of GABA by 50% (IC_{50}) was found to be $2 \cdot 1 \pm 0 \cdot 3$ mM. A possible explanation for the low activity of (4) may be unfavourable steric interactions at both GABA uptake and binding sites. Thus an acidic group at the 3-position of piperidine as in nipecotic acid (1) detracts from activity at receptor binding sites, while an acidic group at the 4-position as in (2) detracts from activity at uptake sites. Accordingly the inactivity of (4) at both binding and uptake sites may result from the fact that (4) has potential carboxyl groups at both the 3- and the 4-positions of the piperidine ring.

Experimental

For general directions see ref.[27] R_F values reported are for thin-layer chromatography (t.l.c.) on Merck Kieselgel 60 precoated t.l.c. plates in butan-1-ol/acetic acid/water (4 : 1 : 1), unless otherwise indicated.

Octahydro-1H-2-pyrindine-5,7-dione (*4*)

Pyridine-3,4-dicarboxylic acid (16·7 g, 100 mmol) was refluxed in acetic anhydride (100 ml) for 15 min and the resultant anhydride cooled to room temperature. Ethyl acetoacetate (13·0 g, 100 mmol) and triethylamine (22·2 g, 220 mmol) were added slowly to maintain the temperature at 45°. Stirring was continued overnight at room temperature and the mixture was then poured

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into ice water (200 ml) with vigorous stirring. The yellow product was filtered, washed with minimal amounts of water, ethanol and ether, and then dried in vacuum to yield the ethoxycarbonyl β-diketo derivative (6), 21·2 g (93%), m.p. 155–160° (dec.) [lit.[14] 170° (dec.) for the compound with half a mole of water of crystallization]. ^{1}H n.m.r. δ [$(CD_3)_2SO$] 9·0, 1H, d, *J* 6 Hz, NCHCH; 8·8, 1H, s, NCH; 7·9, 1H, d, *J* 6 Hz, NCHCH; 4·2, 2H, q, OCH_2; 4·18, s, exchangeable enolic OH; 1·22, 3H, t, CH_3.

Compound (6) (5·5 g, 25 mmol) was dissolved in 1 M sodium hydroxide (25 ml) and water (50 ml) and was reduced on a Parr hydrogenation apparatus over Adams catalyst (300 mg) under 4 atm pressure for 5 h. After decanting the catalyst, this solution was acidified with 6 M hydrochloric acid (about 15 ml) to give a pH of 1·8 and was heated under reflux for 1·5 h. Rotary evaporation of the solvent yielded a crude product (7·3 g) which was absorbed on a column of Dowex 50W (H^+) (75 ml) and eluted with 1 M pyridine. The resultant *octahydro-1H-2-pyrindine-5,7-dione* (4) crystallized from water/isopropyl alcohol (5 ml : 25 ml) to yield 2·2 g (57%), m.p. 225–230° (dec.) (Found: C, 59·3; H, 7·2; N, 8·7. $C_8H_{11}NO_2.0·5H_2O$ requires C, 59·2; H, 7·5; N, 8·6%). ^{1}H n.m.r. (external tetramethylsilane) δ (D_2O) 4·0–3·1, 6H, complex, CH_2NCH_2, CHCH; 2·8–2·1, 2H, m, NCH_2CH_2; ($COCH_2CO$ exchanges rapidly with D_2O). ν_{max} 3480br, 3330br, 2540br, 1620w, 1530s cm^{-1}. λ_{max} (ethanol) 265 nm (log ε 4·45). Mass spectrum (c.i., methane): *m/e* 154 (MH, 100%), 153 (5), 152 (2), 125 (17). pK_a 2·9±0·3; 10·00±0·05 (determined as in ref.[28]). R_F 0·25.

cis-*Piperidine-3,4-dicarboxylic Acid Hydrochloride* (5)

Octahydro-1*H*-2-pyrindine-5,7-dione (4) (107 mg, 0·7 mmol) was dissolved in water (10 ml), sodium periodate (2·25 g, 10·5 mmol) was added and the mixture (pH 4–5) was stirred at room temperature overnight. The product was passed down a column of Dowex 50W (H^+) (50 ml) and removed with 1 M pyridine. Rotary evaporation of the solvent yielded neutral cis-*piperidine-3,4-dicarboxylic acid* (90 mg). This was converted with 6 M hydrochloric acid into the *hydrochloride salt* which crystallized from ethanol to yield (5), 80 mg (55%), m.p. 241–243° (lit.[29] 239°). ^{1}H n.m.r. δ (D_2O) 4·2–3·6, 6H, complex, CH_2NCH_2, CHCH; 2·9–2·45, 2H, m, NCH_2CH_2. ν_{max} 3200(sharp), 1735, 1715 cm^{-1}. R_F 0·18. This product was identical with a sample prepared by hydrogenation of pyridine-3,4-dicarboxylic acid.

t-Butyl 5,7-Dioxooctahydro-1H-2-pyrindine-2-carboxylate (7)

Di-t-butyl dicarbonate (14·8 g, 68 mmol) was added to a solution of the β-diketone (4) in 4 M sodium hydroxide (4 g in 25 ml) and t-butanol (40 ml) and the mixture was warmed to 40–50° for 1 h. After washing with n-hexane (2 × 40 ml), the mixture was acidified dropwise with 6 M hydrochloric acid and extracted into ethyl acetate (3 × 40 ml). The solution was washed with brine, dried with sodium sulfate and the solvent was removed under vacuum. The N-*protected product* (7) crystallized from ethyl acetate (10 ml) yielding 11·3 g (79%), m.p. 162–164° (Found: C, 61·4; H, 7·4; N, 5·5. $C_{13}H_{19}NO_4$ requires C, 61·6; H, 7·6; N, 5·5%). ^{1}H n.m.r. δ ($CDCl_3$) 9·2(broad), OH; 5·4, 1H, s, CH=; 4·3–3·1, 4H, complex; 2·95, 2H, m; 2·1, 2H, m, NCH_2CH_2; 1·45, 9H, s, $(CH_3)_3$. ν_{max} 1690, 1652w, 1565br cm^{-1}. R_F 0·80; 0·57 [ethyl acetate/acetic acid (4 : 1)].

6-Bromooctahydro-1H-2-pyrindine-5,7-dione Trifluoroacetate (8)

A solution of bromine (800 mg, 5 mmol) in water (50 ml) was added to the *N*-protected diketone (7) (1·27 g, 5 mmol) in glacial acetic acid (20 ml) with ice cooling. The mixture was stirred at 0° for 0·5 h and the precipitate was filtered to yield the N-*protected bromo derivative* (11), 1·38 g (83%), m.p. 170–175° (dec.) (Found: C, 47·0; H, 5·3; N, 4·3. $C_{13}H_{18}BrNO_4$ requires C, 47·0; H, 5·5; N, 4·2%). ^{1}H n.m.r. δ [$(CD_3)_2SO$] 6·2(broad), 1H, enolic OH, 4·1–2·7, 6H, complex, CH_2NCH_2, CHCH; 1·95, 2H, m, NCH_2CH_2; 1·35, 9H, s, $(CH_3)_3$. ν_{max} 2600br, 1685, 1550 cm^{-1}. Mass spectrum (c.i., methane): *m/e* 334 (1), 332 (MH, 1%), 306 (5), 304 (6), 278 (85), 276 (100), 198 (33). R_F 0·60.

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Compound (11) (664 mg, 2 mmol) was treated with trifluoroacetic acid (3 ml) at room temperature for 30 min. The solvent was removed under vacuum, azeotropically removed with toluene and the product crystallized from ethanol to give the *trifluoroacetate salt* (8), 382 mg (55%), m.p. 215–225° (dec.). ^{1}H n.m.r. δ (D_2O) 4·1–3·5, 6H, complex, CH_2NCH_2 and CHCH; 2·9–2·3, 2H, m, NCH_2CH_2. ν_{max} 2600br, 1680w, 1550(br) cm^{-1}. λ_{max} (water) 272 nm (log ε 4·39). Mass spectrum (c.i., methane): *m/e* 234 (21), 232 (MH, 21%), 154 (40), 152 (15), 143 (12), 115 (40), 83 (93), 81 (100). R_F 0·28.

*6-Diazooctahydro-1*H*-2-pyrindine-5,7-dione (9)*

4-Toluenesulfonyl azide (1·09 g, 5·5 mmol) and triethylamine (0·56 g, 5·5 mmol) were stirred overnight with a solution of the *N*-protected diketone (7) (1·27 g, 5 mmol) in chloroform (25 ml) at room temperature. The volume was reduced to 5 ml before being chromatographed on a 5-cm column of Kieselgel H (type 60) in a 6-cm diameter sintered glass filter funnel, the eluent being an increasing proportion of ethyl acetate in dichloromethane. Appropriate fractions were combined and removal of solvent yielded the *N*-protected diazo derivative (10) as a yellow oil which crystallized slowly at 4° but melted at less than 20°. ^{1}H n.m.r. δ ($CDCl_3$) 4·25–3·2, 4H, complex; 3·05, 2H, m; 2·15, 2H, m, NCH_2CH_2; 1·45, 9H, s, $(CH_3)_3$. ν_{max} (neat) 2125, 1675 cm^{-1}. Mass spectrum (c.i., methane): *m/e* 280 (MH, 1%) 252 (3), 224 (100), 206 (46), 196 (6), 183 (7), 168 (3). R_F 0·87, 0·64 [n-hexane/ethyl acetate/acetic acid (5 : 4 : 1)].

Compound (10) (100 mg) was deprotected with 45% hydrobromic acid in acetic acid (1 ml). This solution was freeze dried and then recrystallized from methanol to yield *6-diazooctahydro-1H-2-pyrindine-5,7-dione hydrobromide* (9) (40 mg, 43%), m.p. 198–199° (dec. with evolution of gas) (Found: C, 36·6; H, 3·8; N, 16·3. $C_8H_{10}BrN_3O_2$ requires C, 36·9; H, 3·9; N, 16·2%). ^{1}H n.m.r. δ (D_2O) 4·2–3·5, 6H, complex, CH_2NCH_2, CHCH; 2·8–2·2, 2H, m, NCH_2CH_2. ν_{max} 3400, 2150, 1675 cm^{-1}. λ_{max} (ethanol) 244, 217 nm (log ε 4·08, 4·05). Mass spectrum (c.i., methane): *m/e* 180 (MH, 98%), 152 (28), 134 (12), 124 (100), 123 (28), 110 (54), 96 (33), 81 (76).

Biological Assays

Studies were made, by methods previously described, on the 'high affinity' sodium-dependent uptake of GABA,[24] on the activity of GABA:2-oxoglutarate aminotransferase in extracts of rat brain mitochondria,[25] and on the sodium-independent binding of GABA to rat brain membranes;[26] 1 mM GABA was used to correct for non-specific binding.

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