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Descarboxylysergic Acid (9,10-Didehydro-6-methylergoline)

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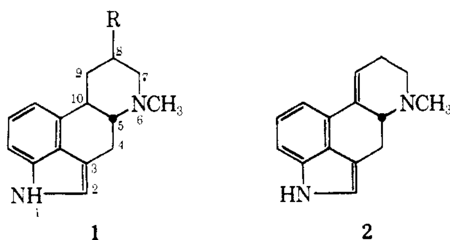
dl-Descarboxylysergic acid (2) has been synthesized by two routes from 9,10-didehydro-2,3-dihydro-8β-hydroxy-6-methylergoline (3). Since 2 shows many of the pharmacological activities of other ergot derivatives, a side chain at the 8 position in an ergoline is *not* essential for biological activity. The unique rigid arylethylamine moiety in this class is probably the key structural feature.

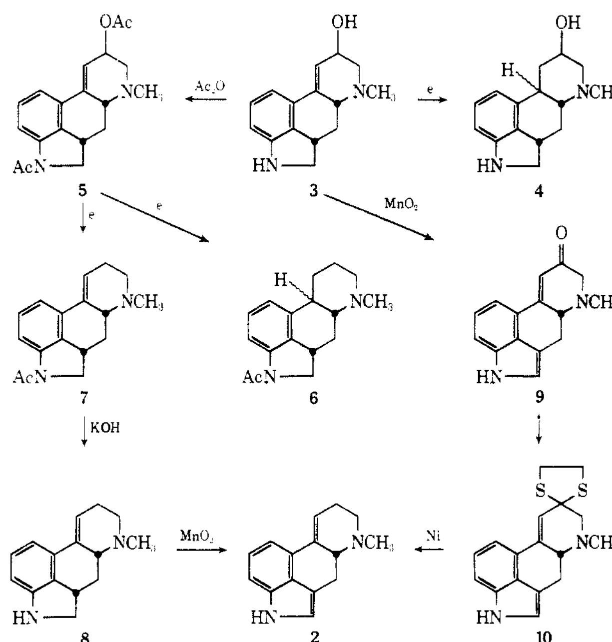
The structures of most of the known ergot alkaloids and their derivatives are based on the tetracyclic ergoline ring system 1 and differ mainly in the character of the side chains (R) at the 8 position. The pharmacology of the various members varies remarkably with the composition of the side chain.[1] Lysergic acid (1, R = COOH, $\Delta^{9,10}$), for instance, has very unexceptional biological activity, while its diethylamide (LSD) (1, R ± $CONEt_2$, $\Delta^{9,10}$) is one of the most potent CNS agents known. Ergonovine [1, R = $CONHCH(CH_3)CH_2OH$, $\Delta^{9,10}$] has profound oxytoxic activity, while other derivatives are serotonin antagonists, vasodilators, hypotensives, prolactin inhibitors,[2,3] etc. With these observations in mind it was of interest to prepare and evaluate the compound in which the side chain at position 8 was absent, *i.e.*, 2 (descarboxylysergic acid). We describe here two synthetic routes to this compound (*dl* form) and record some of its pharmacological properties.



Chemistry (Scheme I). An obvious starting material for the synthesis of 2 was the tetracyclic unsaturated alcohol 3, which was used in our total synthesis of lysergic acid.[4] It is available in nine steps from 3-indolepropionic acid. Initial attempts to cleave the allylic hydroxyl in 3 by electrolytic reduction led only to saturation of the 9,10 double bond to yield the dihydro derivative 4. Similar reductions on the diacetyl derivative 5 were more fruitful. Electrolytic reduction of 5 at a potential of −2.70 V led to both allylic cleavage and saturation of the 9,10 double bond to afford 6. At a potential of −2.30 V, however, the product was the desired 9,10-didehydro-2,3-dihydroergoline (7). Hydrolysis of the acetyl amide function in 7 led to the indoline 8, which was transformed on oxidation with MnO_2 to *dl*-descarboxylysergic acid (2).

An alternative route to 2 was developed by subjecting

Scheme I



the tetracyclic allylic alcohol 3 to MnO_2. Oxidation of both alcohol and dihydroindole functions took place, and the α,β-unsaturated ketone 9 which resulted was converted to the corresponding dithioethylene ketal **10**. This derivative on desulfurization with Raney nickel led in poor yield to *dl*-descarboxylysergic acid (2). In its present state of development, the electrochemical route to 2 is the preferred one.

Pharmacology. In the mouse behavior screen normal male mice were given graded ip injections (0.1 up to 300 mg/kg) of descarboxylysergic acid maleic acid salt to determine the pattern of behavioral effects produced by the drug. The LD_{50} was about 50–100 mg/kg, and the profile of activities observed was remarkably similar to that shown by LSD. However, previous studies have shown that such observations are not reliably predictive of hallucinogenic activity in man.

In isolated smooth muscle studies 2 maleate salt was

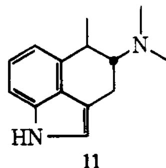
about equivalent to ergonovine maleate salt in contracting isolated rabbit aorta. On isolated rabbit uterus the threshold dose to contract was 1.0 ng/ml (1/10 the potency of the standard drug, ergonovine maleate salt). The ED_{50} of descarboxylysergic acid maleate salt as a serotonin antagonist on the isolated rat stomach strip was 0.03 ng/ml (1/3 the potency of methysergide).

In chloralose anesthetized normal cats **2** maleate salt given iv at doses of 0.1-1.0 mg/kg produced a profound drop in blood pressure. Similarly, in normotensive unanesthetized dogs the compound effected a 21% fall in blood pressure at an iv dose of 0.0625 mg/kg.

Finally, in the rat radioimmunoassay for prolactin inhibition,[2] **2** showed a 45% inhibition at a dose of 100 ng ip. This would indicate that **2** is less than 10% as effective as ergocornine in this test. Further studies with **2** are in progress.

Discussion

From the data presented it is apparent that *dl*-descarboxylysergic acid (**2**) shows many of the responses typical of other ergot derivatives; however, it is usually less active than the best of the standard drugs. Part of this lower potency may be attributed to the racemic nature of **2**. Nevertheless, it is concluded that a side chain at the 8 position is *not* a prerequisite for high biological activity. It would appear that the necessary common structural feature is a rigid arylethylamine, held sterically in the unique conformation **11**, found only in this series. It may be noted that **11** contains *both* a rigid phenethylamine and a tryptamine moiety in a single molecule. The relative importance of these remains to be elucidated.



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Experimental Section

Elemental analyses are indicated only by symbols of the elements and are within 0.4% of the theoretical values. All new compounds were monitored by measurement of ir, uv, and nmr spectra. Mass spectra were determined for most structures and were consistent with the other spectral measurements. Melting points were determined on a Mel-Temp apparatus and are corrected. Tlc was carried out on Merck F254 silica gel plates.

Electroorganic Synthesis Apparatus. The electrolysis instrument was a PAR Model 170 electrochemistry system. The electrolysis cell consisted of a water-jacketed beaker with a ring mercury-pool working electrode, 0.9 cm in width and 5.8 cm in outside diameter. A magnetic stirring bar was located in the center of the ring. The anode compartment was also a ring formed from a halved toroid of slightly smaller dimensions than those of the working electrode. A fine sintered-glass ring disk fused to the toroid was used for separation of compartments. The anode was a platinum wire ring. The reference electrode was a Beckman fiber-junction type containing methanol saturated with potassium chloride. A Beckman fiber-junction bridge containing background electrolyte was used to locate the reference electrode as close to the mercury pool as possible. This cell design, with its parallel anode and cathode, gave the best potential control.

All of the electrolyses were performed with controlled potential for specificity using IR compensation and positive feedback. The high resistance and high initial currents of the solutions resulted in poorly defined voltammograms at the Hg pool. The electrolysis potentials were selected using these voltammograms and polarograms of 1 m*M* solutions of the compound of interest. The background electrolyte in both the anode and cathode compartments was 0.1 *M* tetraethylammonium perchlorate in a 9% by volume water-reagent grade DMF solvent system. Water was used as a proton source. The best water percentage was determined polarographically. The temperature was maintained at 25° throughout. All solutions were shielded from light to prevent decomposition and were deaerated with argon prior to and during electrolysis.

2,3-Dihydro-8β-hydroxy-6-methylergoline (4). Polarographically the starting material **3** showed two reduction steps with half-wave potentials ($E_{1/2}$) of −2.53 and −2.68 V with a current ratio of 4:1, respectively. At the Hg pool only one reduction was discernable at −2.70 V. When the electrolysis was performed at −2.70 V using 0.3 g of **3** in 100 ml of electrolyte, 300 C were passed (theory is 240 for a two-electron transfer). The solution was concentrated *in vacuo*, and the residue was slurried in H_2O and extracted with $CHCl_3$-*i*-PrOH (3:1). The extract was dried (Na_2SO_4) and evaporated to yield 0.158 g (52%) of **4**. A sample was recrystallized from ether: mp 191-195°. *Anal.* ($C_{15}H_{20}N_2O$) C, H, N.

8β-Acetoxy-1-acetyl-9,10-didehydro-2,3-dihydro-6-methylergoline (5). The allylic alcohol **3**[4] (10.0 g) was dissolved in 325 ml of warm Ac_2O, and the solution was stirred for 16 hr. The resulting suspension was poured into water and made basic with $NaHCO_3$. The product was extracted into $CHCl_3$; the extract was washed with H_2O, dried (Na_2SO_4), and concentrated *in vacuo*. The product **5** was crystallized from $CHCl_3$-Et_2O: yield 11.3 g (84%); mp 174-177°. *Anal.* ($C_{19}H_{22}N_2O_3$) C, H, N.

1-Acetyl-2,3-dihydro-6-methylergoline (6). The electrolysis was performed at −2.70 V on a solution of 0.5 g of **5** in 100 ml of electrolyte. The voltammogram was poorly defined. The number of coulombs passed corresponded to a three-electron rather than a four-electron process, indicating the presence of an unwanted side reaction. The solvent was evaporated *in vacuo*, and the residue was slurried with aqueous $NaHCO_3$ and extracted with EtOAc. The extract was washed with H_2O, dried ($MgSO_4$), and evaporated. The product, 0.23 g (55%), was converted to the maleic acid salt, mp 195-196° dec. *Anal.* ($C_{17}H_{22}N_2O{\cdot}C_4H_4O_4$) C, H, N.

1-Acetyl-9,10-didehydro-2,3-dihydro-6-methylergoline (7). Polarographically **5** has two reduction steps with $E_{1/2}$'s of −2.13 and −2.53 V with a current ratio of 3:2, respectively. At a concentration of 2.0 g of **5** in 100 ml of electrolyte the voltammograms at the Hg pool were very poorly defined. At an electrolysis potential of −2.30 V, the number of coulombs passed corresponded to a two-electron transfer. The product was isolated by the procedure used for **6**, yield 1.3 g (78%), characterized as the maleic acid salt, mp 181-182° dec. *Anal.* ($C_{17}H_{20}N_2O{\cdot}C_4H_4O_4$) C, H, N.

9,10-Didehydro-2,3-dihydro-6-methylergoline (8). A solution of 1.12 g of **7**, 5 g of KOH, and 5 ml of 85% hydrazine in 115 ml of ethylene glycol was heated under reflux for 15 hr. It was cooled, diluted with H_2O, and extracted with $CHCl_3$. The extract was dried and evaporated to yield 0.76 g (80%) of crude **8** which was recrystallized from Et_2O-petroleum ether: mp 171-172°. *Anal.* ($C_{15}H_{18}N_2$) C, H, N.

9,10-Didehydro-6-methylergoline (2). A mixture of 1.5 g of **8**, 10 g of activated MnO_2, and 500 ml of $CHCl_3$ was stirred under N_2 for 2.5 hr. The solid was filtered and washed several times with hot $CHCl_3$-EtOH (3:1). The combined filtrates were evaporated *in vacuo*, and the residue was redissolved in $CHCl_3$ and chromatographed on 30 g of Florisil. The product was eluted with $CHCl_3$-EtOH (99:1), yield 0.62 g (40%), and was recrystallized from Et_2O: mp >200° dec. *Anal.* ($C_{15}H_{16}N_2$) C, H, N.

The maleic acid salt was prepared in THF and recrystallized from EtOH: mp 183-185° dec. *Anal.* ($C_{15}H_{16}N_2{\cdot}C_4H_4O_4$) C, H, N.

9,10-Didehydro-6-methylergolin-8-one (9). To a suspension of 2.0 g of **3** in 500 ml of $CHCl_3$ was added 28 g of activated MnO_2. The reaction mixture was stirred for 2 hr, after which time the MnO_2 was filtered and washed several times with hot $CHCl_3$. The combined filtrates were concentrated and then chromatographed on 30 g of Florisil. The product was eluted with $CHCl_3$-EtOH (19:1) and crystallized from Et_2O-hexane: mp 145-148° dec; yield 0.9 g (45%). *Anal.* ($C_{15}H_{14}N_2O$) C, H, N.

9,10-Didehydro-6-methylergolin-8-one Ethylene Dithioketal (10). A suspension of 0.33 g of **9**, 1.0 ml of ethanedithiol, and 0.5 ml of $BF_3{\cdot}Et_2O$ was stirred for 19 hr under N_2. The mixture was taken up in $CHCl_3$-MeOH-H_2O and made basic with $NaHCO_3$. The $CHCl_3$ layer was dried (Na_2SO_4) and concentrated *in vacuo*. The residue was dissolved in $CHCl_3$, and the solution was filtered through Florisil. Removal of the solvent left the product which was crystallized from hexane: mp 190-193° dec; yield 0.31 g (76%). An analytical sample was recrystallized from Et_2O. *Anal.* ($C_{17}H_{18}N_2S_2$) C, H, N, S.

2 by Desulfurization of 9,10-Didehydro-6-methylergolin-8-one Ethylene Dithioketal (10). To a suspension of 7 ml of Raney nickel prepared by the method of Stütz and Stadler[5] in 16 ml of acetone and 4 ml of DMF was added a solution of 0.35 g of the ketal **10** in 15 ml of acetone and 5 ml of DMF. The mixture was stirred for 1 hr, and the nickel was filtered and washed with ace-

tone. The filtrates were diluted with H_2O and extracted with EtOAc. The extract was washed with H_2O, dried (Na_2SO_4), and concentrated *in vacuo*. The residue was chromatographed on 25 g of Florisil. The product was eluted with $CHCl_3$-EtOH (19:1): yield, 20 mg.

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Synthesis and Biological Activity of Some Analogs of the Gonadotropin Releasing Hormone

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Twenty-one analogs of gonadotropin releasing hormone, <Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2 (GnRH), were synthesized by the solid-phase method. The derivatives were <Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-OCH_3 (GnRH methyl ester), GnRH *N*-methyl amide, GnRH free acid, <Glu-His-Trp-Ser-Tyr-Gly-NH_2 (GnRH N-terminal hexapeptide), and also [Ac-Ala[1]]-, [Ac-gly[1]]-, [D-<Glu[1]]-, [Pro[1]]-, [Arg[2]]-, [Tyr(Me)[3]]-, [Ser[5]]-, [des-Gly[6]]-, [Sar[6]]-, [des-Pro[9]]-, [des-Gly[10],Pro-NH_2[9]]-, [des-Gly[10],Pro-NHC_2H_5[9]]-, [Tyr[11]]-, [Tyr(Me)[3,5]]-, [des-His[2],des-Pro[9]]-, [des-His[2],Sar[6]], and [Sar[6]·des-Tyr[5]]-GnRH. All analogs were purified at both the protected and the deblocked stage. The final products were characterized by chemical and physical methods and assayed *in vitro* for both LH and FSH release using rat pituitaries. Those derivatives which showed less than 0.2% of the activity of GnRH itself were further tested for inhibition of gonadotropin release.

There have now appeared several descriptions of the total synthesis of the gonadotropin releasing hormone (GnRH),[1-6] as well as of GnRH analogs.[7-13] In a collaborative project with Takeda Industries, Ltd., the effects of simple substitution of several of the amino acids in the GnRH molecule with other naturally occurring amino acids have been studied.[12] In a recent report the effect of modification of an amide function on proline in position 9 has been explored. From these efforts have emerged several noteworthy agonists in the [des-Gly[10]] series,[13] one of which demonstrates up to five times the activity of GnRH. These results are indeed encouraging, but more elusive goals such as finding an antagonist have yet to be met. In the work reported here an attempt has been made to eliminate agonist activity by making a variety of substitutions, deletions, and other structural changes in the hope of finding an antagonist.

The procedures used in this laboratory for the preparation of GnRH[6,14] were based chiefly on the solid-phase method.[15] These same procedures have provided a means of producing GnRH analogs with the following advantages: (a) the rapidity and versatility of synthesizing analogs by the solid-phase method, (b) standardization of methods for isolating the protected GnRH analogs by a general purification method involving silica gel column chromatography, and (c) purification of the analogs by the general method of gel filtration on Sephadex. Examples of the purification methods employed are shown in Figures 1-4. The silica gel column chromatography (first pass) of the methyl ester III shown in Figure 1 clearly demonstrates the removal of impurities encountered in the solid-phase preparation. After a second pass (not shown), the product was obtained as a single spot on tlc (R_f's of Table I). This purification was typical of all analogs prepared and, in fact, more than half required only one purification by silica gel column chromatography. Figures 2 and 4 show the isolation of a single product on Sephadex G-25 for a large preparation of GnRH-free acid (VIII, 769 mg) and a moderate size preparation of [Ac-Gly[1]]-GnRH (XIV, 143 mg). After passage through the first Sephadex G-25 column most of the analogs showed a single spot by tlc (R_f's of Table II). Figure 3 demonstrates the use of Sephadex G-15 to improve the quality of VIII (1.0 mg) which had been previously obtained by chromatography on Sephadex G-25.

The use of nmr spectra (Tables III and IV) has been a particularly convenient tool in following structural modification of the GnRH molecule. Some general statements can be made to describe the spectra. <Glu was difficult to observe but a peak centered at 2.4 ppm due to the $-CH_2$ group was visible as a broadening at the base of the Tos-CH_3 singlet. The Tos group was easily determined by the resonance of the aromatic proton at 8.68 ppm. Trp was somewhat difficult to detect but generally showed aromatic proton resonance at 7.6 ppm and also a 7.02-ppm singlet attributable to the hydrogen at position 1. Ser(Bzl) was easily seen by the singlet resonance of the Bzl-CH_2 at 4.47 ppm. In the absence of the blocking group, the presence of Ser could be determined by the integral of the 3.5-4.2-ppm region. Tyr(Bzl) was also easily seen by the Bzl-CH_2 proton at 4.93 ppm and also by the aromatic resonances (½ AA^1BB^1 multiplet) centered at 6.87 ppm. The number of Gly present could be obtained from the integral in the 4.0-ppm region which was due to the CH_2 resonances. Leu was very easily determined by $(CH_3)_2$ resonances (two doublets) at 0.9 ppm. The presence of Arg(Tos) could be detected by the occurrence of a CH_3 singlet at 2.36 ppm and also by aromatic resonance at 7.75 ppm. Pro had no easily detected resonances.

Other physical data include melting points (Table I), yields (Tables I and II), optical rotations (Table I and II), and amino acid analysis (Tables V and VI). The biological