# Notes

## Potential Central Nervous System Antineoplastic Agents. Amides of 6-Cyano-6-norlysergic Acid

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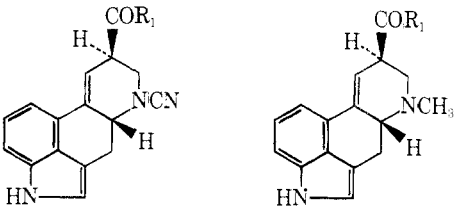
A group of amides of 6-cyano-6-norlysergic acid was prepared from their corresponding lysergamides. None of these *N*-cyano derivatives showed anticancer activity against lymphoid leukemia L1210 in mice. This is in contrast to the reported in vitro anticancer activity (P-815 mastocytoma) of 6-cyano-6-norlysergic acid diethylamide.

Lysergic acid derivatives display marked effects on the central nervous system (CNS) which are a complex mixture of excitation and depression.[1a] Recently they (e.g., ergocornine) have been shown to be potent inhibitors of prolactin secretion and hold some promise as therapeutic agents in the treatment of hormone dependent mammary tumors.[1b] In the course of our program to prepare potential CNS antineoplastic agents, we were therefore very interested in a recent patent literature report of cytostatic activity exhibited by 6-cyano-6-norlysergic acid derivatives.[2] These included the methyl ester **1a** and, in particular, the 6-cyano-6-norlysergic acid diethylamide [**1**, $R_1 = N(Et)_2$] which was reported to cause 50% inhibition of mouse P-815 mastocytoma in vitro at 5–6 mg/l. If the in vitro activity of these compounds could be confirmed against lymphoid leukemia L1210 in vivo as a primary screen, these findings could serve as a lead to prepare other *N*-cyanonorlysergamides as potential CNS antineoplastic agents. The main objective of our study was therefore to (a) confirm the activity of these 6-cyano-6-nor compounds in vivo and (b) synthesize and test other related compounds in order that a functional group–activity relationship might be defined in this series. The results of this study are described in this paper.

We have found that the methyl ester **1a** is inactive against L1210 under a variety of doses (in vivo). To confirm our finding, we prepared other *N*-cyanonorlysergamides and found that none of them showed anticancer activity (T/C >125) in this test. The lack of activity of these compounds against L1210 in vivo is in contrast to the reported[2] in vitro activity of **1a** in P-815. This discouraged us from preparing other derivatives in this series.

**Chemistry.** Amides of *d*-lysergic acid were prepared by the method of Johnson and Ary[3] by the reaction of lysergic acid with the appropriate amine in the presence of phosphorus oxychloride. The mono- (**2**, $R_1 = NH$-*n*-Bu)[3] and di-*n*-butylamides (**2c**)[3] were isolated as their maleate salts whereas the amide **2b** was isolated as the free base[4] after column chromatography on silica gel since the maleate salt[5] failed to crystallize. *d*-Lysergic acid on treatment with methanolic HCl gave the methyl ester **2a**[6] which was isolated either as the maleate salt or as the free base.

To prepare the 6-cyano-6-nor derivatives of the amides **1b–d** and the methyl ester, the maleate salts were converted to the free bases[3] with $NH_4OH$ and subsequently treated with cyanogen bromide in methylene chloride.[7,8] The final cyano compounds were dark colored and showed tendency to decompose in the presence of air and light. It is interesting to note that the secondary amide **2** ($R_1 = NH$-*n*-Bu) failed to give the *N*-cyano product under these experimental conditions, and the material obtained could not be characterized.




1 2

a, R = $OCH_3$
b, R = N⬡
c, R = $N(n\text{-Bu})_2$
d, R = N△

**Biological Results.** The compounds were tested in mice against L1210 according to standard protocol[9] by the screening contractors of the National Cancer Institute. The test compounds were administered at 400, 200, 100, 50, 25, 12.5, and 6.25 mg/kg intraperitoneally in sonified saline with polysorbate 80. Doses were administered beginning on day 1, every fourth day for three doses, and test results were evaluated at day 30. Compound **1b** was also tested on a regimen beginning on day 1, daily for nine doses. Evaluations were made as mean survival time and compounds showing percent test/control (T/C) > 125 against L1210 are considered active in this test system.

Compounds **1a–c** were found to be inactive in this test showing a maximum T/C of 100 (at a 50 mg/kg dose), 119 (6.25 mg/kg), and 101 (100 mg/kg), respectively.

### Experimental Section

All melting points are uncorrected and were determined in an evacuated capillary on a Thomas-Hoover apparatus. All compounds showed ir, NMR, and mass spectra consistent with the assigned structures. Microanalyses were performed by the Heterocyclic Chemical Corp., Harrisonville, Mo.

The *N*-cyano derivatives were prepared by the general procedure as described in the preparation of **1c**.

***d*-*N*,*N*-Di(*n*-butyl)-6-cyano-6-norlysergamide (1c).** To a magnetically stirred solution of 3.1 g (8.2 mmol) of **2c**[3] (as the free base) in 50 ml of dry $CH_2Cl_2$ contained in a round-bottom flask cooled with an ice bath was added 4.63 g (44.1 mmol) of cyanogen bromide followed by 100 ml of dry $CH_2Cl_2$. The flask was swept briefly with dry nitrogen and stoppered. The resulting dark yellow solution was stirred at ambient temperature for 4.5 hr, resulting in a blue-green mixture, which was evaporated under reduced pressure leaving a blue-green residue. After cooling the flask in an ice bath, 100 ml of 2 *N* tartaric acid was added followed by 200 ml of $CH_2Cl_2$. Upon shaking, an emulsion formed making phase interface difficult to discern. A 200-ml portion was drawn off as the $CH_2Cl_2$ phase and the remaining aqueous phase was extracted twice with 100 ml of $CH_2Cl_2$. The combined organic phase was

washed twice with 200 ml of deionized water, dried ($Na_2SO_4$), filtered, and concentrated on a rotary evaporator (25°) to give 3.067 g of a dark red residue. Column chromatography on 50 g of silica gel (Woelm 0.05–0.20 mm) and elution with 0.5% $C_2H_5OH$ in $CHCl_3$ gave 2.1 g of a green crystalline solid: ir (film) 2240 (C≡N, strong), 1640 $cm^{-1}$ (C=O amide, strong); TLC (10% MeOH–$CHCl_3$ on silica gel) showed one spot, $R_f$ 0.7. The solid was recrystallized from approximately 5 ml of 1:1 $i$-$Pr_2O$–$CH_2Cl_2$ to yield 0.921 g (29% yield) of bronze-colored crystals: mp 81–85° dec; mass spectrum (70 eV) $m/e$ 390 ($M^+$), 347, 333, 307. Anal. ($C_{24}H_{30}N_4O$) C, H, N.

***d*-6-Cyano-6-norlysergic Acid Methyl Ester (1a).**[8] The method described above was used except that after work-up the crude product was crystallized directly ($i$-$Pr_2O$–$CH_2Cl_2$, 37% yield) and no column chromatography was carried out: mp 138–140° (lit.[8] 146°).

**1-(*d*-6-Cyano-6-norlysergoyl)piperidine (1b).** This compound was prepared as 1c in 15% yield. It was crystallized from $i$-$Pr_2O$–$CHCl_3$: mp 114–117°; mass spectrum (70 eV) $m/e$ 346 ($M^+$), 262, 234, 233, 207. Anal. ($C_{21}H_{22}N_4O$) C, H, N.

**1-(*d*-6-Cyano-6-norlysergoyl)pyrrolidine (1d).**[10] Compound **2d**[11] was treated with BrCN as in 1c. After work-up the crude material was purified by two preparative TLC separations on 2-mm thick silica gel plates (Merck, 10% $CH_3OH$–$CHCl_3$) and crystallized from a mixture of $CHCl_3$–$Et_2O$: mp 220–223° (40% yield); mass spectrum (70 eV) $m/e$ 332 ($M^+$), 262, 234, 207, 193, 192.

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## References and Notes

(1) (a) L. S. Goodman and A. Gilman, "The Pharmacologic Basis of Therapeutics", Macmillan, New York, N.Y., 1970, pp 557, 893; (b) see, for example, J. Meites and J. A. Clemens, *Vitam. Horm. (N.Y.)*, **30**, 165–221 (1972); H. G. Floss, J. M. Cassady, and J. E. Robbers, *J. Pharm. Sci.*, **62**, 699 (1973).
(2) T. Fehr and P. Stadler, Swiss Patent 535,235; *Chem. Abstr.*, **79**, 32162b (1973).
(3) F. N. Johnson and I. E. Ary, *J. Med. Chem.*, **16**, 532 (1973).
(4) The free base (48% yield) was crystallized from isopropyl ether, mp 114–115°.
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(9) (a) R. I. Geran, N. H. Greenberg, M. M. MacDonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep.*, **3** (2), 1 (1972); (b) Instruction Booklet 14, "Screening Data Summary Interpretation", Drug Research and Development, Chemotherapy, National Cancer Institute, Bethesda, Md., 1972.
(10) The quantity of material prepared was insufficient for biological testing.
(11) British Patent 785,173; *Chem. Abstr.*, **52**, 7365 (1958). For the maleate salt, see ref 5.

# Studies in Antifertility Agents. 11. Secosteroids. 5. Synthesis of 9,11-Secoestradiol[1]

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9,11-Secoestradiol (**9**) and 11-hydroxy-9,11-secoestradiol (**12**) have been synthesized starting from 17-acetoxyestradiol 3-methyl ether (1) and found to possess significant antifertility activity in rats. 3-Methoxy-9,11-seco-9-oxo-17β-acetoxyestra-1,3,5(10)-trien-11-oic acid (**2**), prepared by $CrO_3$ oxidation of 1, on hydrogenolysis gave methyl 17β-hydroxy-3-methoxy-9,11-secoestra-1,3,5(10)-triene-11-carboxylate (**3**). The 17-O-THP derivative of **3** was treated with $LiAlH_4$ to give 17β-(*O*-tetrahydropyranyl)-3-methoxy-11-hydroxy-9,11-secoestra-1,3,5(10)-triene (**5**). The 11-*O*-mesylate of **5** on $LiAlH_4$ reduction followed by mild acid treatment and demethylation under alkaline conditions gave **9**. $LiAlH_4$ reduction of **3** gave 9,11-seco-11-hydroxyestradiol 3-methyl ether (11) which on demethylation gave 9,11-seco-11-hydroxyestradiol (**12**).

In a search for atypical estrogens (substances with dissociated activity of estrone) possessing specific antifertility, hypocholesteramic, or antiinflammatory activity and as probes for study of "estrogenic" receptors, secoestrones have been under investigation in this laboratory for sometime.[2] A recent communication by Chinn et al.[3] describing the synthesis of secoequilenin prompted us to describe our work on 9,11-secoestradiol and some related compounds. In order to ensure estradiol stereochemistry in the compounds it was considered practical to start with estradiol and carry out chemical operations on it which are not likely to affect the stereochemistry of the nucleus.

3-Methoxy-17β-acetoxyestra-1,3,5(10)-triene (**1**) on $CrO_3$ oxidation gave 3-methoxy-9,11-seco-9-oxo-17β-acetoxyestra-1,3,5(10)-trien-11-oic acid (**2**),[4] which on hydrogenolysis gave methyl 17β-hydroxy-3-methoxy-9,11-secoestra-1,3,5(10)-triene-11-carboxylate (**3**). The 17-tetrahydropyranyl derivative of **3** on lithium aluminum hydride reduction gave 17β-(*O*-tetrahydropyranyl)-3-methoxy-11-hydroxy-9,11-secoestra-1,3,5(10)-triene (**5**). The 11-*O*-mesylate **6**, prepared from **5**, on lithium aluminum hydride reduction followed by acid treatment to remove the tetrahydropyranyl group and demethylation[5] with potassium hydroxide and hydrazine hydrate in diethylene glycol gave the desired 9,11-secoestradiol (**9**). Alkaline hydrolysis of **3** gave 3-methoxy-17β-hydroxyestra-1,3,5(10)-trien-11-oic acid (**10**). 11-Hydroxyestradiol (**12**) was obtained from **3** by lithium aluminum hydride reduction to **11** followed by demethylation under alkaline conditions.

**Biological Activity.** Compounds **9–12** were tested for their antifertility activity in pregnant female albino rats of proven fertility. The day on which the vaginal smears showed the presence of spermatozoa was considered day 1 of pregnancy. In the primary screening the compounds were fed for 5 days from day 1 to day 5 of pregnancy using five animals in each group. The compounds were macerated with gum acacia and administered orally to animals. The results were scored as positive only if implantations were totally absent in both uterine horns, examined on day 10 of pregnancy; control animals had an average of seven