A New Approach to 16α-Hydroxycorticoids[1]

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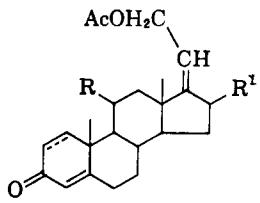
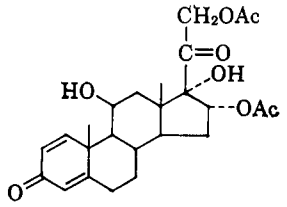
A novel method of synthesis for 16α-hydroxycorticoids is described.

The 16α-hydroxyl group has become an important substituent in cortical hormones.[2] Three general methods have been described for the preparation of 16α-hydroxycorticoids. In the first method a 16-pregnen-20-one was hydroxylated with osmium tetroxide[2a-c] or potassium permanganate,[2e] while in the second method the 16α-hydroxyl was introduced by microbiological oxidation,[2d] and in the third method 16β-bromo-17α-acetates were treated with acetoxylating reagents or mild bases.[2f]

Allylic oxidation[3] of 11β,21-dihydroxy-1,4,17(20)-*cis*-pregnatrien-3-one 21-acetate[4] (**1**) with selenium dioxide in aqueous dioxane introduced the 16α-hydroxyl group to form **2** in 66% yield and a minor amount of ketone **3**. The allylic nature of the newly introduced hydroxyl group was established by quantitative oxidation with manganese dioxide to the corresponding ketone (**3**). The ultraviolet and infrared absorption spectra of **3** indicated that the new hydroxyl group of **2** was at position 16. Unequivocal proof of structure was obtained by acetylation of the 16-hydroxyl group followed by oxidation with osmium tetroxide-*N*-methylmorpholine oxide peroxide[5] to yield 11β,16α,17α,21-tetrahydroxy-1,4-pregnadiene-3,20-dione 16,21-diacetate (**4**).[2c] An authentic sample of **4** was prepared by osmium tetroxide hydroxylation of 11β,21-dihydroxy-1,4,16-pregnatriene-3,20-dione 21-acetate (**5**)[6] followed by acetylation. The identity of the two samples established that selenium dioxide oxidation introduced a 16α-hydroxy group into 11β,21-dihydroxy-1,4,17(20)-*cis*-pregnatrien-3-one 21-acetate (**1**). Since compounds similar to **1** have been described containing 6α-methyl,[7] 6α-fluoro,[8] 2α-methyl,[9] and 2α-fluoro[10] substituents; selenium dioxide oxidation of these compounds offers a convenient route to the correspondingly substituted 16α-hydroxy compounds useful intermediates in the synthesis of the corresponding corticoids. This transformation in the 6α-methyl and 6α-fluoro series is reported elsewhere.[11]





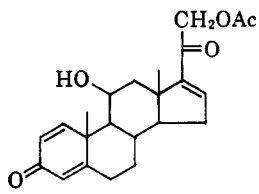
4

Δ[1] present

1, R = OH; R[1] = H
2, R = OH; R[1] = - - - OH
3, R = OH; R[1] = =O

Δ[1] absent

6, R = OH; R[1] = H
7, R = OH; R[1] = - - - OH
10, R = O; R[1] = H
11, R = O; R[1] = - - - OH
12, R = R[1] = =O



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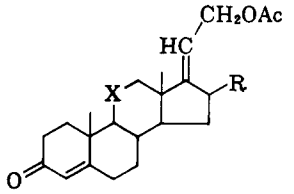
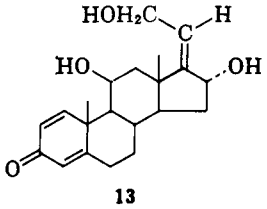


8, R = H
9, R = - - - OH
9a, R = =O
} X = >C(OH)H

10a, R = H
11a, R = - - -OH
} X = >C=O



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The contributions to the molecular rotation made by the 16-hydroxyl group in the 17(20)-*cis* series, −118° (*cf.* **10** and **11**), and the 17(20)-*trans* series, −97° (*cf.* **10a** and **11a**), are consistent with the assignment of the α-orientation of that group[12] in both series (*cf.* Table I).

Selenium dioxide allylic oxidation of 11β,21-dihydroxy-4,17(20)-*trans*-pregnadien-3-one 21-acetate (**8**) gave modest yields of both the 16α-hydroxy and 16-keto compounds **9** and **9a**. Since **8** had the thermodynamically preferred *trans* configuration at the 17(20)-positions, it can be assumed that its oxidation product **9**

(1) For a preliminary report of a portion of this material, see B. J. Magerlein, R. D. Birkenmeyer, and F. Kagan, *J. Am. Chem. Soc.*, **82**, 1252 (1960).

(2) (a) W. S. Allen and S. Bernstein, *ibid.*, **78**, 1909 (1956); (b) S. Bernstein, R. H. Lenhard, W. S. Allen, M. Heller, R. Littell, S. M. Stoller, L. I. Feldman, and R. H. Blank, *ibid.*, **78**, 5693 (1956); (c) **81**, 1689 (1959); (d) R. W. Thoma, J. Fried, S. Bonanno, and P. Grabowich, *ibid.*, **79**, 4818 (1957); (e) G. Cooley, B. Ellis, F. Hartly and V. Petrow, *ibid.*, 4373 (1955); (f) J. Romo and A. Romo DeVivar, *J. Org. Chem.*, **21**, 902 (1956).

(3) O. Rosenheim and W. W. Starling, *J. Chem. Soc.*, 377 (1937).

(4) J. A. Hogg, F. H. Lincoln, A. H. Nathan, A. R. Hanze, B. J. Magerlein, W. P. Schneider, P. F. Beal, and J. Korman, *J. Am. Chem. Soc.*, **77**, 4438 (1955).

(5) W. P. Schneider and A. R. Hanze, U. S. Patent 2,769,823 (1956).

(6) F. Kagan, B. J. Magerlein, and R. D. Birkenmeyer, *J. Org. Chem.*, **28**, 3477 (1963).

(7) G. B. Spero, J. L. Thompson, B. J. Magerlein, A. R. Hanze, H. C. Murray, O. K. Sebek, and J. A. Hogg, *J. Am. Chem. Soc.*, **78**, 6213 (1956).

(8) J. A. Hogg, G. B. Spero, J. L. Thompson, B. J. Magerlein, W. P. Schneider, D. H. Peterson, O. K. Sebek, H. C. Murray, J. C. Babcock, R. L. Pederson, and J. A. Campbell, *Chem. Ind.* (London), 1002 (1958).

(9) J. A. Hogg, F. H. Lincoln, R. W. Jackson, and W. P. Schneider, *J. Am. Chem. Soc.*, **17**, 6401 (1955).

(10) A. H. Nathan, B. J. Magerlein, and J. A. Hogg, *J. Org. Chem.*, **24**, 1517 (1959).

(11) B. J. Magerlein, F. H. Lincoln, R. D. Birkenmeyer, and F. Kagan, *Chem. Ind.*, (London) 2050 (1961).

(12) The values for M_D 16αOH-H range from −44 to −82°. *Cf.* D. K. Fukishima and T. F. Gallagher, *J. Am. Chem. Soc.*, **73**, 196 (1951); and D. Perlman, E. Titus, and J. Fried, *ibid.*, **74**, 2126 (1952). The average value for M_D 16βOH-H is +38° (*cf.* D. K. Fukishima and T. F. Gallagher).

TABLE I

MOLECULAR ROTATION DIFFERENCES (ΔMD) FOR 16-HYDROXY STEROIDS

Pair	Compound	[α]D (acetone), deg.	MD, deg.	MD16αOH-H, deg.
10	21-Hydroxy-4,17(20)-*cis*-pregnadiene-3,11-dione 21-acetate	+155	+574	−118
11	16α,21-Dihydroxy-4,17(20)-*cis*-pregnadiene-3,11-dione 21-acetate	+118	+456	
10a	21-Hydroxy-4,17(20)-*trans*-pregnadiene-3,11-dione 21-acetate	+142	+526	−97
11a	16α,21-Dihydroxy-4,17(20)-*trans*-pregnadiene-3,11-dione 21-acetate	+111	+429	

retained this stereochemistry. Since **9** was not identical to **7**, the oxidation product obtained in the *cis* series, it is likely that the *cis* configuration at C-17(20) is unaltered during the hydroxylation. Nuclear magnetic resonance data provided further evidence to confirm this hypothesis. Slomp[13] has shown that a *trans* configuration about a $\Delta^{17,20}$-double bond provides a greater shielding effect on the C-18 protons than the corresponding *cis* isomer by approximately 6–12 c.p.s. (at 60 Mc.). An examination of the C-18 proton resonance frequencies for a group of $\Delta^{17,20}$-olefins and their 16α-hydroxy derivatives, prepared by selenium dioxide allylic oxidation (*cf.* Table II) shows that, in all of the cases examined, the chemical shifts for the C-18 protons in the oxidation products occurred at frequencies comparable to that of the starting materials, whereas comparison of *cis–trans* pairs, such as **10** and **10a** and **11** and **11a**, illustrates the difference in shielding effects exerted on the C-18 protons by the *cis*- and *trans*-17,20-olefinic side chains.

TABLE II

C-18 PROTON RESONANCE IN *cis* AND *trans* $\Delta^{17,20}$-OLEFINS AND THEIR SELENIUM DIOXIDE OXIDATION PRODUCTS

Pair	Compound	C-18 proton frequency (c.p.s. at 60 Mc. relative to TMS)
10	21-Hydroxy-4,17(20)-*cis*-pregnadiene-3,11-dione 21-acetate	55
11	16α,21-Dihydroxy-4,17(20)-*cis*-pregnadiene-3,11-dione 21-acetate	55
10a	21-Hydroxy-4,17(20)-*trans*-pregnadiene-3,11-dione 21-acetate	49
11a	16α,21-Dihydroxy-4,17(20)-*trans*-pregnadiene-3,11-dione 21-acetate[a]	48
1	11β,21-Dihydroxy-1,4,17(20)-*cis*-pregnatrien-3-one 21-acetate	69[b]
2	11β,16α,21-Trihydroxy-1,4,17(20)-*cis*-pregnatrien-3-one 21-acetate	72
8	11β,21-Dihydroxy-4,17(20)-*trans*-pregnadien-3-one 21-acetate	61[b]
9	11β,16α,21-Trihydroxy-4,17(20)-*trans*-pregnadien-3-one 21-acetate	. .

[a] Prepared by G. B. Spero of these laboratories. [b] Converted from 40-Mc. spectrum.

Finally, selenium dioxide oxidation of 11β,21-dihydroxy-4,17(20)-*cis*-pregnadien-3-one 21-acetate (**6**), and its 11-keto analog (**10**) afforded in good yield their respective 16α-hydroxy analogs (**7** and **11**). 1-Dehydrogenation of **7** with *Septomyxa affinis*[14] produced triol **13** identical by paper chromatographic analysis with that obtained by alkaline hydrolysis of **2**.

(13) Private communication from G. Slomp, Physical and Analytical Chemistry Department, The Upjohn Co.

Experimental[15]

11β,16α,21-Trihydroxy-1,4,17(20)-*cis*-pregnatrien-3-one 21-Acetate (2).—In a 500-ml. three-necked flask, fitted with a stirrer, reflux condenser, and a thermometer, were placed 9.8 g. (0.026 mole) of 11β,21-dihydroxy-1,4,17(20)-*cis*-pregnatrien-3-one 21-acetate (**1**), 2.76 g. (0.025 mole) of selenium dioxide, 33 ml. of water, and 150 ml. of dioxane. This mixture was stirred and heated at reflux for 1 hr. The reaction mixture was cooled in an ice bath to about 25°, 5.0 g. of Magnesol[16] was added, and, after stirring for an additional 15 min. the mixture was filtered under vacuum through a Magnesol mat. The filtrate was poured into 900 ml. of methylene chloride, and this solution was then washed with four 200-ml. portions of water. The methylene chloride phase was filtered and evaporated to a volume of about 100 ml., and the product was isolated *via* chromatography over 800 g. of Florisil.[17] The column was developed by eluting with acetone–Skellysolve B[18] mixtures, the acetone concentration gradually being increased until the crystalline product fraction was obtained at an eluting concentration of 20 to 25% acetone. After one recrystallization from acetone–Skellysolve B the product melted at 178–181°, 6.74 g. (66% yield). Four recrystallizations gave material, $[\alpha]_D$ +83° (chloroform); λ_{max}^{EtOH} 243 mμ (ε 15,600); m.p. 179–181°; n.m.r. (c.p.s.), 72, 122, doublet at 284 (J = 3), triplet at 338 (J = 7), doublet of doublets at 371, 373, 381, and 383, and doublet at 441 (J = 5).

Anal. Calcd. for $C_{23}H_{30}O_5$: C, 71.48; H, 7.82. Found: C, 71.68; H, 8.07.

A second crystalline product (0.4% yield), m.p. 261–263°, which was eluted from the column in the early 20% acetone fractions, was shown to be identical by mixture melting point with 11β,21-dihydroxy-1,4,17(20)-*cis*-pregnatriene-3,16-dione 21-acetate (**3**) prepared as described in the next section.

11β,21-Dihydroxy-1,4,17(20)-*cis*-pregnatriene-3,16-dione 21-Acetate (3).—In a 250-ml. flask were placed 1.3 g. (3.4 mmoles) of 11β,16α,21-trihydroxy-1,4,17(20)-*cis*-pregnatrien-3-one 21-acetate (**2**), 5.0 g. (0.75 mole) of activated manganese dioxide,[19] and 140 ml. of ethyl acetate. The flask was stoppered and shaken at about 25° for 17 hr. The reaction mixture was filtered and the filtrate evaporated, yielding 1.25 g. (97% yield) of a white solid. The analytical sample, $[\alpha]_D$ −15° (chloroform), λ_{max}^{EtOH} 241.5 mμ (ε 24,400), was recrystallized from acetone and melted at 264–266°.

Anal. Calcd. for $C_{23}H_{28}O_5$: C, 71.85; H, 7.34. Found: C, 71.81; H, 7.45.

11β,16α,17α,21-Tetrahydroxy-1,4-pregnadiene-3,20-dione 16,21 Diacetate (4). A. From 11β,16α,21-Trihydroxy-1,4,17(20)-*cis*-pregnatrien-3-one 21-Acetate (2).—Six grams of 11β,16α,21-trihydroxy-1,4,17(20)-*cis*-pregnatrien-3-one 21-acetate (**2**) was treated with 30 ml. of acetic anhydride and 30 ml. of pyridine at 26° for 17 hr. Methylene chloride was added and the mixture washed successively with dilute hydrochloride acid, water, and sodium bicarbonate solution. An oil was obtained on evaporation of the solvent. This oil resisted crystallization even after chromatography over Florisil and was used directly in the following step.

A solution of 2.0 g. of crude diacetate, 2 ml. of pyridine, 40 mg. of osmium tetroxide, 5.8 ml. of *N*-methylmorpholine oxide peroxide, and 60 ml. of *t*-butyl alcohol was stirred at 26° for 17 hr.

(14) The authors are indebted to H. C. Murray of these laboratories for biooxidation and identification of the product.

(15) Melting points were taken in capillary tubes. Rotations were observed at 26°. N.m.r. spectra were run by G. Slomp on a Varian DP 60 spectrometer at 60 Mc. and calibrated against internal tetramethylsilane (as zero) using audiofrequency side-band interpolations. The n.m.r. spectra of compounds **10**, **10a**, **11**, and **11a** were run on a Varian A-60 spectrometer.

(16) Magnesium silicate formerly manufactured by Westvaco-Chlor-Alkali Division, Food, Machinery and Chemical Corp., New York.

(17) A synthetic magnesia–silica gel manufactured by the Floridin Co., Warren, Pa.

(18) A saturated hydrocarbon fraction, b.p. 60–71°.

(19) J. Attenburrow, A. F. B. Cameron, J. H. Chapman, R. M. Evans, B. A. Hems, A. B. A. Jansen, and T. Walker, *J. Chem. Soc.*, 1094 (1952).

A dilute solution of sodium hydrosulfite was added in slight excess. The butyl alcohol was distilled *in vacuo*, and the residue partitioned between methylene chloride and dilute hydrochloric acid. The methylene chloride layer was percolated through 150 g. of Florisil. The oily fraction eluted with Skellysolve B-15% acetone was identified by its infrared spectrum as starting diacetate and amounted to a 35% recovery. The more polar fraction of about 700 mg., eluted with Skellysolve B–50% acetone, was recrystallized from ethyl acetate–Skellysolve B to yield 170 mg. of **4**, m.p. 142–149° (solvated). Further recrystallization gave solvated crystals melting at 156–163° and 162–165°.

B. From 11β,21-Dihydroxy-1,4,16-pregnatriene-3,20-dione 21-Acetate (5).—In a dry, 50 ml. one-necked flask were placed 1.19 g. (3.1 mmoles) of 11β,21-dihydroxy-1,4,16-pregnatriene-3,20-dione 21-acetate (**5**), 25 ml. of benzene, 1.2 ml. of pyridine, and 785 mg. (3.1 mmoles) of osmium tetroxide. After standing at 25° for about 100 hr. the dark brown reaction mixture was poured into a solution of 55 ml. of water, 20 ml. of benzene, 36 ml. of methanol, 5.6 g. of sodium sulfite, and 5.6 g. of potassium bicarbonate and stirred for 4 hr. This mixture was extracted six times with 100-ml. portions of hot chloroform; the combined extract was washed with dilute hydrochloric acid and then with water. After drying over sodium sulfate and evaporation of the chloroform, 670 mg. of a tan solid was obtained. Recrystallization from methanol gave 230 mg. (17% yield) of 11β,16α,17α,21-tetrahydroxy-1,4-pregnadiene-3,20-dione 21-acetate as a methanol solvate; $[\alpha]_D$ +67° (chloroform); λ_{max}^{EtOH} 243 mμ (ε 14,300); m.p. 222–224°.

Anal. Calcd. for $C_{23}H_{30}O_7$: C, 66.01; H, 7.23. Found: C, 66.15; H, 7.60 (dried at 120°).

Acylation of 150 mg. of this material with acetic anhydride–pyridine, yielded after recrystallization from ethyl acetate 95 mg. of **4**, m.p. 158–163°, whose infrared spectrum was identical with a sample of **4** prepared according to method A.

11β,16α,21-Trihydroxy-4,17(20)-*cis*-pregnadien-3-one 21-Acetate (7).—A mixture of 3.7 g. of 11β,21-dihydroxy-4,17(20)-*cis*-pregnadien-3-one 21-acetate (**6**) and 1.10 g. of selenium dioxide in 55 ml. of dioxane and 15 ml. of water was heated under reflux for 1 hr. The solution was filtered to remove the selenium. The filtrate was diluted with water and extracted with methylene chloride. Concentration of the methylene chloride solution gave 4.5 g. of a yellow oil. Crystallization from ethyl acetate afforded 2 g. of **7**, m.p. 172–177°. The analytical sample, m.p. 179.5–181°, was prepared by chromatography over alumina followed by recrystallization from ethyl acetate.

Anal. Calcd. for $C_{23}H_{32}O_5$: C, 71.10; H, 8.30. Found: C, 71.10; H, 8.14.

21-Hydroxy-4,17(20)-*cis*-pregnadiene-3,11,16-trione 21-Acetate (12).—To a solution of 600 mg. of **7** in 8 ml. of acetic acid was added 500 mg. of sodium dichromate dihydrate; the solution was stirred for 2 hr. at room temperature. Water was added and the resulting crystalline precipitate was collected by filtration, washed with water, and dried. The yield of crude product was 200 mg. melting at 177–181°. This material was chromatographed over Florisil. The main fraction, eluted with 7.5–10% acetone in Skellysolve B, was recrystallized several times from ethyl acetate–Skellysolve B. It melted at 191–194°; $[\alpha]_D$ +32° (chloroform); λ_{max}^{EtOH} 238 mμ (ε 24,750).

Anal. Calcd. for $C_{23}H_{28}O_5$: C, 71.85; H, 7.34. Found: C, 71.59; H, 7.04.

16,21-Dihydroxy-4,17(20)-*cis*-pregnadiene-3,11-dione 21-Acetate (11).—A mixture of 14.5 g. of 21-hydroxy-4,17(20)-*cis*-pregnadiene-3,11-dione 21-acetate (**10**), 215 ml. of dioxane, 50 ml. of water, and 4 g. of selenium dioxide was heated under reflux for 1 hr. After cooling the reaction mixture to 26°, 5.5 g. of Magnesol was added, and the mixture was stirred for 20 min. The solids were removed by filtration and the filtrate diluted with 1 l. of methylene chloride. The organic solution was washed with water six times. Raney nickel, previously washed with methanol was added to the solution, and the mixture was stirred for 20 min. After filtering through Magnesol the solution was dried over sodium sulfate. The solution was concentrated to dryness and the crude product crystallized from ethyl acetate. The crystals, melting at 233–236° and weighing 6.9 g. (45%), were collected by filtration. The analytical sample, m.p. 245–246°, $[\alpha]_D$ +145° (chloroform), was recrystallized from ethyl acetate.

Anal. Calcd. for $C_{23}H_{30}O_5$: C, 71.48; H, 7.82. Found: C, 71.23; H, 7.53.

11β,16α,21-Trihydroxy-1,4,17(20)-*cis*-pregnatrien-3-one (13).—A solution of 1.82 g. of **2** in 60 ml. of methanol and 60 ml. of 0.1 *N* sodium hydroxide was maintained at 26° for 18 hr. The reaction mixture was concentrated to about 30 ml. and diluted with water. The crude product, 1.6 g., was recovered by filtration. A portion, recrystallized twice from methanol, melted at 224–244°; $[\alpha]_D$ +65° (chloroform).

Anal. Calcd. for $C_{21}H_{28}O_4$: C, 73.22; H, 8.19. Found: C, 73.24; H, 8.43.

Microbial 1-Dehydrogenation of 11β,16α,21-Trihydroxy-4,17(20)-*cis*-pregnadien-3-one 21-Acetate (7).—A fermentation mixture containing 10 l. of a medium consisting of 200 g. of liquid corn steep water and 100 g. of commercial dextrose adjusted to pH 5.0 with 25% aqueous sodium hydroxide was sterilized. This medium was inoculated with 5% of a 72-hr. vegetative growth in *Septomyxa affinis* grown in a shake flask. After aerating at 0.5 l. of air per min. and agitating vigorously for 24 hr., 1.25 g. of **7** was added in 50 ml. of dimethylformamide. After an additional 20 hr. of agitation and aeration, the pH had risen to 8.6. The 10 l. of filtered culture medium was extracted four times with one-fourth volume of methylene chloride. The solvent was evaporated and an aliquot indicated (by paper chromatography with a benzene–formamide system) that 25% of starting **7** remained and the 11β,16α,21-trihydroxy-1,4,17(20)-*cis*-pregnatrien-3-one 21-acetate was produced to the extent of 25% of the starting steroid.

11β,16α,21-Trihydroxy-4,17(20)-*trans*-pregnadien-3-one 21-Acetate (9) and 11β,21-Dihydroxy-4,17(20)-*trans*-pregnadiene-3,16-dione 21-Acetate (9a).—A solution of 3.5 g. of 11β,21-dihydroxy-4,17(20)-*trans*-pregnadien-3-one 21-acetate (**8**),[20] 1.5 g. of selenium dioxide, 33 ml. of water, and 75 ml. of dioxane was heated under reflux for 1 hr. The solvent was distilled under vacuum and the residue extracted with methylene chloride. Chromatography over Florisil using Skellysolve B with increasing proportions of acetone led to the recovery of 1.1 g. of **8** and two more polar crystalline fractions. The less polar fraction weighed 230 mg. and melted at 149–153°. The analytical sample prepared from ethyl acetate–Skellysolve B melted at 156–157° and had $[\alpha]_D$ +15° (chloroform); λ_{max}^{EtOH} 240 mμ (ε 25,050). The infrared spectrum was in accord with structure **9a**.

Anal. Calcd. for $C_{23}H_{30}O_5$: C, 71.48; H, 7.82. Found: C, 71.32; H, 7.91.

The more polar fraction was recrystallized from ethyl acetate to yield 170 mg. of **9**, m.p. 200–205°. Several recrystallizations gave an analytical sample, m.p. 215–217°; $[\alpha]_D$ +132° (chloroform).

Anal. Calcd. for $C_{23}H_{32}O_5$: C, 71.10; H, 8.30. Found: C, 70.69; H, 8.89.

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(20) Private communication from P. F. Beal of these laboratories.