hydrogenated over platinum oxide (0.5 g) at 45 psi for 5 hr. The catalyst was filtered, and the filtrate was evaporated to dryness under reduced pressure to give a white powder. The hydrochloride salt thus obtained was dissolved in 30 ml of water and extracted twice with ether. The aqueous layer was made basic with 10% NaOH, saturated with sodium chloride, and extracted three times with ether. The ether extracts were combined, dried over sodium sulfate, and evaporated to dryness to give 5.6 g (75%) of pale-yellow oil with an NMR spectrum identical to that described for the product from Method A.

3,4,5-Trihydroxybenzylamine Hydroiodide (V)—To a magnetically stirred solution of IV (7.5 g, 38 mmoles) in 47% hydroiodic acid (75 ml, 470 mmoles) under nitrogen was added, by syringe, 43.5 ml (460 mmoles) of acetic anhydride through a septum. The reaction mixture was refluxed for 4 hr and evaporated to dryness *in vacuo*. Ethanol (100 ml) was added to the residue, and the solvent was evaporated again to yield a pale-yellow powder (5.0 g, 46%). Recrystallization from ethanol plus ether gave fine crystals, mp 196–198° dec.; NMR (deuterium oxide): 4.00 (s, 2H, CH_2) and 6.55 (s, 2H, aromatic).

Anal.—Calc. for $C_7H_9NO_3$·HI: C, 29.68; H, 3.53; N, 4.96. Found: C, 29.66; H, 3.52; N, 4.81.

2,3,4-Trimethoxybenzylamine (VI)—Compound VI was prepared as an oil in a 73% yield on a 5-g scale from the amide, using borohydride reduction as described for IV. It was used without further purification; NMR (deuterochloroform): 1.95 (s, 2H, NH_2), 3.70 (s, 2H, CH_2), 3.79–3.83 (m, 9H, CH_3), 6.50 (d, 1H, J = 4.5 Hz, aromatic), and 6.85 (d, 1H, J = 4.5 Hz, aromatic).

2,3,4-Trihydroxybenzylamine Hydroiodide (VII)—Compound VII, mp 178–181° dec., was prepared on a 7-g scale (89%) from VI by the procedure used for V; NMR (deuterium oxide): 4.15 (s, 2H, CH_2), 6.50 (d, 1H, J = 4.5 Hz, aromatic), and 6.81 (d, 1H, J = 4.5 Hz, aromatic).

Anal.—Calc. for $C_7H_9NO_3$·HI: C, 29.68; H, 3.53; N, 4.96. Found: C, 29.81; H, 3.70; N, 4.87.

2,3-Dimethoxybenzylamine (X)—2,3-Dimethoxybenzonitrile (3.29 g, 20 mmoles) was dissolved in 200 ml of absolute ethanol, and 10 ml of chloroform and 0.5 g of platinum oxide were added to this solution. The mixture was hydrogenated for 3 hr at 40 psi, the catalyst was filtered, and the solvent was evaporated. Then the residue was dissolved in a minimum amount of water and made basic with 10% NaOH. The mixture was saturated with sodium chloride and extracted three times with ether. The ether extracts were combined, dried over sodium sulfate, and evaporated to dryness to give 2.9 g (90%) of a yellow oil. The oil was used without further purification; NMR (deuterochloroform): 2.25 (s, 2H, NH_2), 3.8 (m, 8H, CH_2 and CH_3), and 6.81 (m, 3H, aromatic).

2,3-Dihydroxybenzylamine Hydroiodide (XI)—Compound XI, mp 157–158° dec., was prepared from X on a 5-g scale (60%) by the procedure used for V; NMR (deuterium oxide): 4.20 (s, 2H, CH_2) and 6.95 (m, 3H, aromatic).

Anal.—Calc. for $C_7H_9NO_2$·HI: C, 31.46; H, 3.75; N, 5.24. Found: C, 31.59; H, 3.96; N, 5.26.

Antitumor Testing—Antitumor activity was determined as percent T/C values, with T/C ≥ 125% defined as statistically significant (Table I). Dose–response studies were carried out for each compound according to published National Cancer Institute protocols (10). Six mice per dose were inoculated intraperitoneally with 10^6 P-388 leukemia cells on Day 0. Control (untreated) mice usually died on about Day 11. Mice receiving drug were treated on Days 1–9 with intraperitoneal doses of the compound under investigation. Physiological saline (0.9%) was the vehicle. The mice (average weight 20 g) were weighed on Day 5; the weight difference between treated and control mice (T − C) was an indication of dose toxicity. Weight losses greater than 4 g were considered excessive.

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Aluminum Chlorohydrate III: Conversion to Aluminum Hydroxide

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Received October 3, 1980, from the **Department of Industrial and Physical Pharmacy and the* ‡*Department of Agronomy, Purdue University, West Lafayette, IN 47907.* Accepted for publication December 8, 1980.

Abstract □ Bayerite, an aluminum hydroxide polymorph, readily forms when the hydroxyl to aluminum ratio of aluminum chlorohydrate is raised to 3 by titration with sodium hydroxide. Dilution of aluminum chlorohydrate solutions with water leads to the formation of gibbsite, another aluminum hydroxide polymorph. The mechanism of conversion in each instance is related to the structure of the $Al_{13}O_4(OH)_{24}(H_2O)_{12}^{7+}$ complex.

Keyphrases □ Aluminum chlorohydrate—conversion to aluminum hydroxide □ Aluminum hydroxide—conversion from aluminum chlorohydrate □ Antiperspirant activity—conversion of aluminum chlorohydrate to aluminum hydroxide

Aluminum chlorohydrate recently was shown (1) to be the $Al_{13}O_4(OH)_{24}(H_2O)_{12}^{7+}$ (I) complex described by Johansson *et al.* (2). This structure is unusual in that it is composed of a central aluminum atom in tetrahedral configuration surrounded by 12 octahedral aluminum atoms. The charge is neutralized by chloride ions. In contrast, aluminum hydroxide is composed exclusively of aluminum in octahedral configuration. The basic unit of aluminum hydroxide is a six-member ring composed of aluminum in octahedral configuration formed by a dehydration–deprotonation reaction (3). The relationship between aluminum chlorohydrate and aluminum hydroxide was demonstrated by examining the effect of neutralizing aluminum chlorohydrate by the addition of sodium hydroxide and the effect of diluting with water.

EXPERIMENTAL

Aluminum chlorohydrate was obtained commercially as a 50% (w/w) solution[1]. The effect of increasing the hydroxyl to aluminum ratio of aluminum chlorohydrate to 3 was studied by adding 500 ml of 0.394 *N* NaOH at a rate[2] of 10 ml/min to 500 ml of an aluminum chlorohydrate

[1] Lot 8473, Wicken Products, Huguenot, N.Y.

[2] Buchler Polystaltic Pump, Buchler Co., Fort Lee, N.J.

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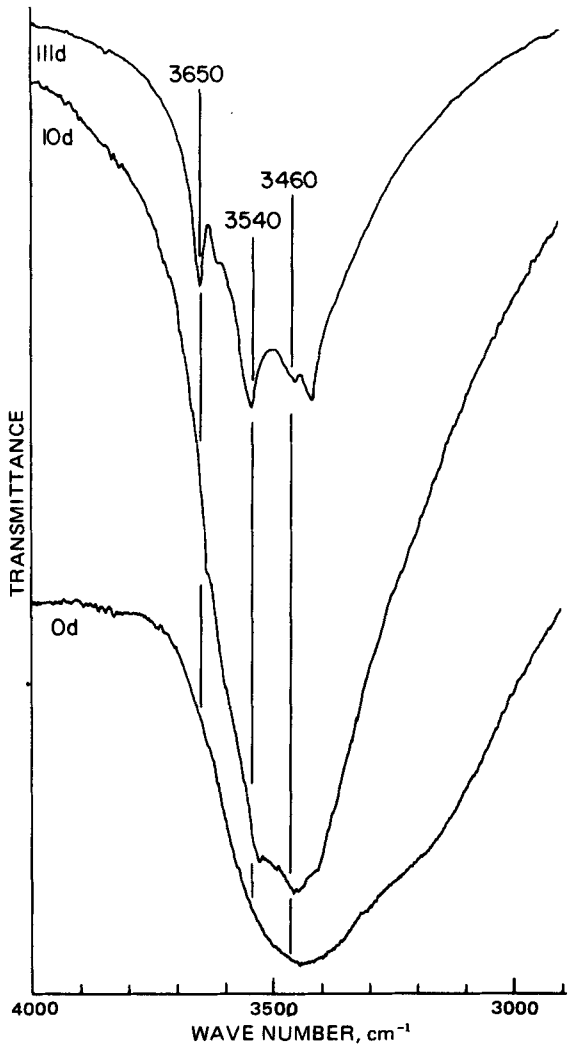

Figure 1—*Change in hydroxyl-stretching frequency region of IR spectrum following neutralization of aluminum chlorohydrate. Key: bottom curve, initial; middle curve, after 10 days at 25°; and top curve, after 111 days at 25°.*

solution, which was 0.788 *M* in aluminum. Upon completion of the titration, stirring was continued for 10 min; the solid phase that formed was washed with distilled water. After air drying, the solid phase was examined by X-ray diffraction[3] and IR spectroscopy[4].

The effect of dilution with deionized water was studied by adding enough distilled water to the commercial 50% (w/w) aluminum chlorohydrate solution (6.2 *M* in aluminum) to produce solutions ranging from 1.39×10^{-3} to 2.5 *M* in aluminum. The solutions were aged at 25° in plastic bottles. Turbidity was monitored by measuring the absorbance at 400 nm. The white precipitate that formed in some solutions was collected by filtration through a 100-Å filter[5]. The solid phase was washed with distilled water and lyophilized. A sample of the filtrate also was lyophilized. Both lyophilized samples were examined by IR spectroscopy as potassium bromide pellets.

RESULTS AND DISCUSSION

The relationship between aluminum chlorohydrate and aluminum hydroxide was investigated by titrating an aluminum chlorohydrate solution with sodium hydroxide until the hydroxyl to aluminum ratio was 3. Figure 1 shows the hydroxyl-stretching region of the IR spectrum before and after titration. After 10 days, this region was much sharper, and indications of distinct absorption bands had developed. After 111 days,

[3] Siemens A. G. Kristalloflex 4 generator, type F diffractometer, Karlsruhe, West Germany.
[4] Model 180, Perkin-Elmer Corp., Norwalk, Conn.
[5] Filter VFWP 047-00, Millipore Corp., Bedford, Mass.

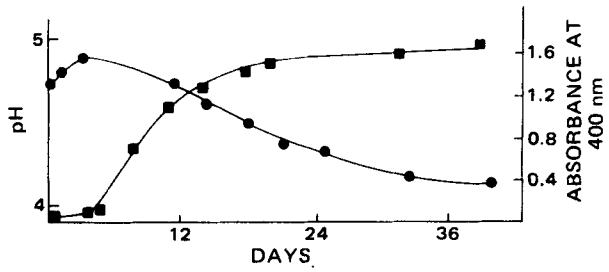

Figure 2—*Change in pH (●) and turbidity (■) following dilution of aluminum chlorohydrate to 2.5×10^{-2} M in aluminum during aging at 25°.*

sharp bands occurred at 3650, 3540, and 3460 cm^{-1}. These hydroxyl-stretching bands correspond to the hydroxyl-stretching vibrations of bayerite (4). The X-ray diffractogram of the 111-day sample had sharp peaks at 4.72, 4.35, 3.20, 2.22, and 1.72 Å. The position of these peaks also correspond to those of bayerite (4).

The conversion of aluminum chlorohydrate to bayerite can be rationalized based on the structure of the I complex. The added hydroxyl

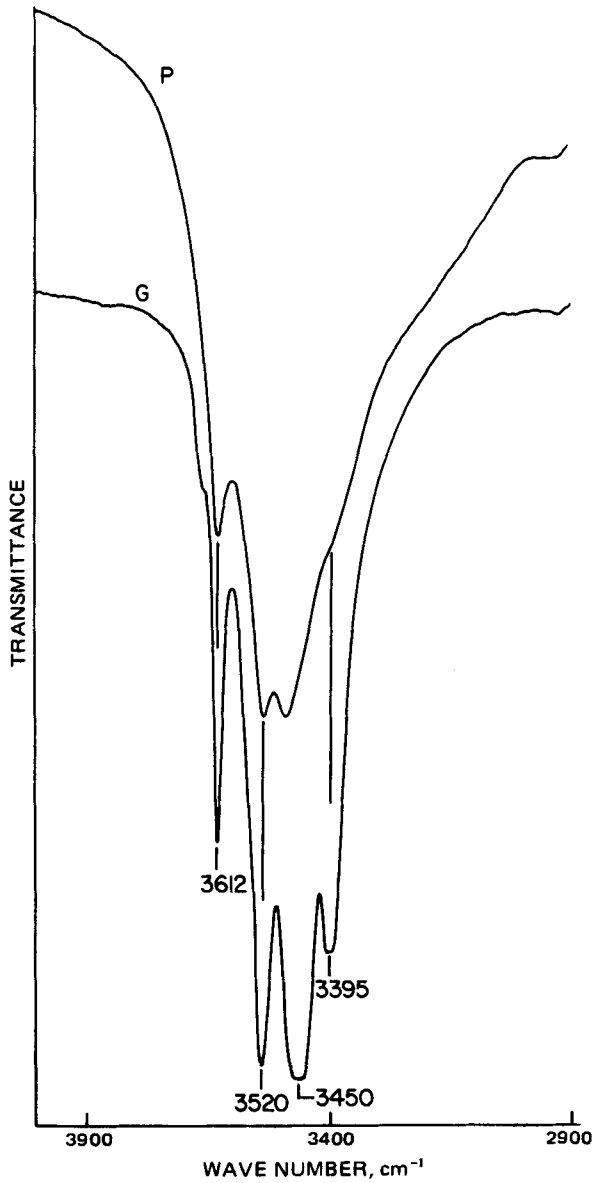

Figure 3—*Hydroxyl-stretching frequency region of IR spectrum of solid phase that formed following dilution of aluminum chlorohydrate to 2.5×10^{-2} M in aluminum (P) compared to natural gibbsite (G).*

anions are strongly attracted to the highly charged aluminum polynuclear cation. As the charge is neutralized, the complex disassociates and aluminum hydroxide forms. Because the solution pH was raised to 8 by the addition of sodium hydroxide, bayerite, the aluminum hydroxide polymorph that forms under basic conditions, was produced.

Aluminum chlorohydrate also is unstable if it is diluted with water (5, 6). Figure 2 shows that when aluminum chlorohydrate was diluted to 2.5×10^{-2} *M* in aluminum, the pH first increased and then decreased. Earlier reports only noted a decrease in pH following dilution. However, the initial increase in pH is important and is consistent with the recently proposed structure of aluminum chlorohydrate (1). The central tetrahedral aluminum in the I complex is surrounded by 12 aluminum atoms in octahedral configuration. In dilute aqueous solution, the highly charged aluminum polycations are widely separated, and the stabilizing effect of the chloride counterions and other aluminum chlorohydrate complexes is reduced. As the I complex disassociates, the tetrahedral aluminum is exposed to the aqueous environment. Since the octahedral configuration is the stable form of aluminum below pH 8 (7–10), the tetrahedral aluminum converts to an octahedral configuration. Exposure of the four negatively charged oxygens, which form the outer shell of the aluminum tetrahedral, to the aqueous environment results in the attraction of protons and water to complete the octahedral configuration. The adsorption of protons is reflected in the initial increase in pH. However, the overall trend is a decrease in pH, which is consistent with the formation of octahedral aluminum into aluminum hydroxy polymers by the deprotonation–dehydroxylation mechanism (3).

The stability of aluminum chlorohydrate is concentration dependent since very little change in pH was observed unless the solution was <0.1 *M* in aluminum. At high concentration, the positively charged I spherical complexes are tightly packed and surrounded by stabilizing chloride counterions. Thus, aluminum chlorohydrate complex is self-stabilizing at high concentration.

Turbidity measurements of the diluted aluminum chlorohydrate solutions also showed that the stability of the complex was concentration dependent. Turbidity was not observed during this study unless the concentration was ~0.1 *M* or lower in terms of aluminum. Figure 2 also shows the development of turbidity following dilution to 2.5×10^{-2} *M* in aluminum.

The insoluble degradation product resulting from the dilution of aluminum chlorohydrate was collected by filtration and examined by IR spectroscopy. The hydroxyl-stretching bands coincide with the hydroxyl-stretching bands of gibbsite (Fig. 3). The IR spectrum of the lyophilized filtrate was identical to the reference spectrum for aluminum chlorohydrate (11). Thus, it is believed that the I complex disassociates in dilute solution. The tetrahedral aluminum converts to an octahedral configuration due to the pH, and the octahedral aluminum species form aluminum hydroxide by deprotonation–dehydration. Gibbsite is the end-product of dilution since it is the stable polymorph of aluminum hydroxide in the acidic region. Therefore, the conversion of aluminum chlorohydrate to aluminum hydroxide upon complete neutralization or following dilution with water is consistent with the structure of the I complex that was proposed recently to be aluminum chlorohydrate.

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Modified Colorimetric Method for Plasma Prednisolone

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Received October 27, 1980, from the *College of Pharmacy and Allied Health Professions, St. John's University, Jamaica, NY 11439.* Accepted for publication January 30, 1981.

Abstract □ A simple, precise, rapid, and sensitive colorimetric method was adapted for the quantitative analysis of prednisolone in dog plasma. A paper chromatographic procedure was modified by the use of thin-layer plates coated with microcrystalline cellulose. Heparinized blood samples were separated from the cellular elements and extracted with methylene chloride. After washing with acid and alkali, the extract was evaporated to dryness. The residue was dissolved in methanol and streaked on the TLC plate. After development, the band that was detected by UV light was scraped off and extracted with methanol. The methanol extract was treated with the Porter–Silber reagent (phenylhydrazine). Absorbance was measured at 410 nm. Replicate assays indicated a mean recovery of 97.5% and a coefficient of variation of 5.13%.

Keyphrases □ Prednisolone—modified method for colorimetric determination in dog plasma □ Colorimetry—modified method for determination of plasma prednisolone □ Glucocorticoids—prednisolone, colorimetric determination in plasma

The various methods used for the determination of prednisolone in plasma all have one or more shortcomings. Jenkins and Sampson (1) used a paper chromatographic procedure for the isolation of prednisolone after preliminary extraction with methylene chloride. Their procedure was modified by replacing the paper chromatographic procedure with thin-layer plates coated with microcrystalline cellulose. Microcrystalline cellulose was tried after preliminary screening revealed that inefficient separation occurred on the silica gel-coated plates recommended in the USP XX procedure (2).

The modified procedure decreased the time required for separation and also increased assay sensitivity. As a consequence, a simple, direct, and rapid analytical method for prednisolone in plasma was developed independently.

EXPERIMENTAL

Materials—Anhydrous prednisolone USP[1], methanol[2] (HPLC grade),

[1] Merck & Co., Rahway, N.J.
[2] Fisher Scientific Co., Fair Lawn, N.J.

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