45.7%). This latter fraction was saponified with ethanolic KOH and 4-ethynylborneol (m.p. 122°, $[\alpha]_D$ +22°(in EtOH, l=10 cm., c=20)) was obtained.

(7) With 90% HCOOH : A mixture of 10 g. of the sample and 60 cc. of 90% HCOOH was refluxed at 100° for 6 hr. and the reaction mixture treated as usual. The fractional distillation gave a fraction of b.$p_{2.5}$ 100~101°, which was saponified with ethanolic KOH to yield 4-ethynylborneol, m.p. 120°, $[\alpha]_D$ +20°(1 g.; yield, 10%); and a fraction of b.$p_{3.5}$ 110~113°, which was saponified with ethanolic KOH to yield 4-acetylborneol, b.$p_{3.5}$ 130~135° (6.7 g., yield, 67%); semicarbazone, m.p. 205°.

Summary

1) The epimers of 2-ethynylborneol were resolved by means of alumina adsorption. They behaved similarly to an anionotropic rearrangement, forming the same product.

2) 2-Ethylborneol was dehydrated in exocyclic way with hydrochloric acid or formic acid to form 2-ethylidenecamphane. 2-Ethylcamphene, which is presumably formed by endocyclic dehydration, was not found.

3) 2-Ethynylborneol yielded aldehyde or ketone by the action of hydrochloric acid or formic acid, respectively. These reactions were studied under varying conditions in order to determine the mechanisms of the rearrangement.

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135. Kyoji Hayano and Sataro Imado : Studies on Aluminum Complex Compound of PAS. I. Synthesis of Alumino-*p*-aminosalicylic Acid*[2] and its Calcium Salt.*[3]

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The rôle of *p*-aminosalicylic acid (hereinafter abbreviated as PAS) as a chemotherapeutic is of value but PAS preparations now in use, such as PAS, PAS-sodium, and basic PAS-calcium, have many defects. PAS itself has an acid taste, PAS-sodium is bitter, and both have unpleasant taste on taking. Further, they are liable to cause gastrointestinal disturbances such as loss of appetite, vomiting, and diarrhoea. Therefore, basic PAS-calcium is more increasingly used than PAS-sodium at present, but even this is not tasteless and gastrointestinal disturbance is not rare. Improvement of PAS preparations to remove such defects has long been desired.

Recently, Deeb and others[1] reported that a preparation of PAS utilizing buffer action of aluminum hydroxide gives very little gastrointestinal disturbance and maintains effective blood level over a long period. Based on this idea, a new compound in which aluminum is bonded to PAS was prepared in order to improve the foregoing defects in PAS preparations, whicn is described herein.

1. Alumino-*p*-aminosalicylic Acid (Al-PAS)

Past literature on a compound of PAS and aluminum is found only in the report of

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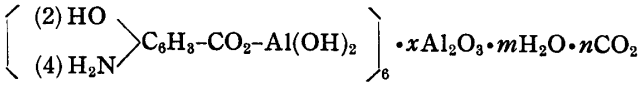
*2 Chemical term : Bis(*p*-aminosalicylato)aquahydroxoaluminatic acid trihydrate or heptahydrate. Abbreviated here as Al-PAS.

*3 Chemical term : Calcium bis(*p*-aminosalicylato)aquahydroxoaluminate heptahydrate. Abbreviated here as Al-PAS-Ca.

1) E. N. Deeb, G. R. Vitagliano : Am. Rev. Tuberc., **72**, 543(1955).

Brown and Beekman[2] on basic aluminum *p*-aminosalicylate which they obtained by the reaction of PAS with basic aluminum carbonate and they gave the following structure to it.



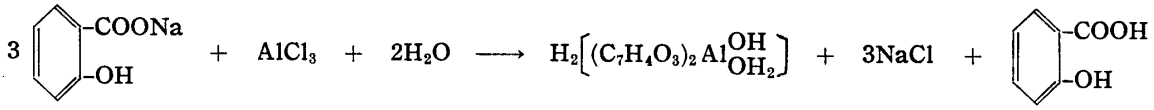
$x=3\sim6$, m, n=not critical

It is clear from the above formula that this substance is not a compound with a definite composition because x, m, and n in the formula vary with the amount of PAS and basic aluminum carbonate used, and degree of drying of the product. Reëxamination of this reaction by the present writers also gave the same result. The ratio of aluminum to PAS in the said substance is 2 or more and the PAS content is less than 37.7%, which means that a large quantity must be taken for therapeutic purpose and this is undesirable in a pharmaceutic.

On the other hand, Burrows and William carried out studies on compounds of salicylic acid and aluminum, and obtained a complex compound of the chemical formula $H_2[Al(C_7H_4O_3)_2(OH)(H_2O)]$. This forms a salt $M_2^I[Al(C_7H_4O_3)_2(OH)(H_2O)]$ by neutralization with alkali or by double decomposition.

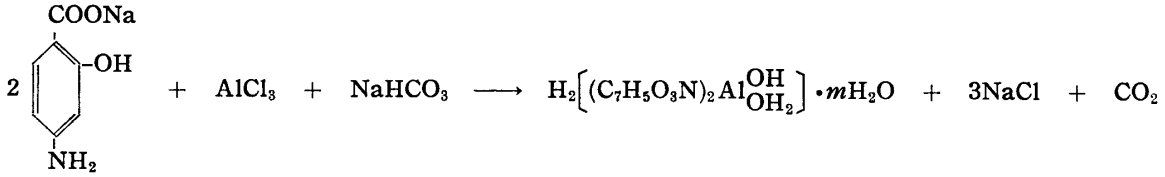
Burrows and others considered that HO and H_2O bonded to aluminum are coordinated to 1 and 6 positions of the central atom and that the structure of $[Al(C_7H_4O_3)_2(OH)(H_2O)]^{2-}$ is a molecular asymmetry and should be resolvable to optical antipodes. However, they have not succeeded in its resolution.

The same reaction was carried out using PAS in place of salicylic acid, according to the method of Burrows, and alumino-*p*-aminosalicylic acid was prepared. The reaction followed the course shown below :



The free salicylic acid formed in this reaction is likely to be contaminated in alumino-salicylic acid and Burrows removed this acid by washing the reaction product with ethanol.

In the case of PAS-sodium and aluminum chloride, similarly liberated PAS would be sparingly soluble not only in ethanol but in most of other solvents that it would be difficult to remove this PAS. In order to avoid this difficulty and also to better the yield, one mole of sodium hydrogen carbonate was added to the reaction mixture so as to neutralize the liberated hydrochloric acid. As a result, the reaction proceeded quite smoothly and Al-PAS was obtained in a very good yield.



The product dried on a porcelain plate is a hexahydrate ($m=6$) but changes to trihydrate ($m=3$) when dried at 50°.

This substance comes as a colorless, fine crystalline powder. It has a faintly sweet taste at first and somewhat astringent later but is far more easier to take than the PAS

2) P.M. Brown, Jr., M. Beekmann : C.A., **48**, 14131(1954); U.S. Pat. 2,686,800(1954).
3) G.J. Burrows, I. William : J. Chem. Soc., **1928**, 222.

preparations now in use. It is sparingly soluble in water and in most of organic solvents like methanol, ethanol, and benzene, and is weakly acid. Exactly one mole of Al-PAS dissolves in ammonia water containing 2 moles of ammonia to form a clear solution. It also dissolves in a solution of 2 moles of sodium hydroxide but the solution is labile and a white precipitate of aluminum hydroxide separates after some time.

2. Calcium Alumino-*p*-aminosalicylate (Al-PAS-Ca)

Illari[4] prepared aluminosalicylic acid by a method different from that of Burrows by application of $KAl(SO_4)_2 \cdot 12H_2O$ or $Al(NO_3)_3 \cdot 9H_2O$ to salicylate. Since the aluminum content of its product was low, he tried drying the aluminosalicylic acid so obtained for a long period of time at 100°, 120°, and 140°, but found that, instead of decrease in water, the amount of salicylic acid decreased and the product changed completely to (*o*)HO-C_6H_4-$COOAl(OH)_2$. When the latter was refluxed with an excess of salicylic acid in the presence of water, aluminosalicylic acid was formed and Illari concluded that aluminosalicylic acid is an equimolar addition compound of (*o*)HO-C_6H_4-$COOAl(OH)_2$ and salicylic acid, and that the structure of the complex compound $H_2\left[(C_7H_4O_3)_2Al\begin{matrix}OH\\OH_2\end{matrix}\right]$ forwarded by Burrows and others is wrong.

However, Burrows and others isolated crystals of sodium aluminosalicylate, $Na_2\left[(C_7H_4O_3)_2Al\begin{matrix}OH\\OH_2\end{matrix}\right] \cdot H_2O$, and also obtained its dihydrate from aluminosalicylic acid by a different route, as well as a barium salt, $Ba\left[(C_7H_4O_3)_2Al\begin{matrix}OH\\OH_2\end{matrix}\right] \cdot H_2O$, sparingly soluble in water by addition of aqueous solution of barium chloride to the above sodium salt.

For the purpose of examining the difference of opinion of Illari and Burrows, and to synthesize a new compound, preparation of Al-PAS-Ca was attempted by the method of Burrows and others. Prior to this experiment, conductometric titration of Al-PAS hexahydrate was carried out and it was found that it is a dibasic acid and that the value agreed well with theoretical value calculated as a hexahydrate.

As was stated above, Al-PAS dissolves in 2 moles of sodium hydroxide but undergoes decomposition after some time to form a white precipitate of aluminum trihydroxide, while it dissolves in 2 moles of ammonia to form a clear solution. Application of calcium chloride solution in equivalent amount to this clear solution afforded colorless crystals in a good yield. This substance is tasteless and odorless, is sparingly soluble in water and in organic solvent, and corresponds to a heptahydrate of the calcium salt, $Ca\left[(C_7H_5O_3N)_2Al\begin{matrix}OH\\OH_2\end{matrix}\right] \cdot 7H_2O$.

PAS content of this substance is around 57%, close to that of 60% in basic PAS-calcium now being used as a tuberculosis drug, and also contains calcium which is thought to be a requisite in the treatment of tuberculosis. It should especially be noted that this substance is completely tasteless.

Result of clinical experiments[5] has shown that administration of Al-PAS-Ca in patients who cannot take basic PAS-Ca decreases gastrointestinal disturbances and that the blood level of PAS rises gradually after administration of Al-PAS-Ca, the high level being maintained over a comparatively long period, in contrast to the rapid rise of blood level of PAS and short duration of this level after administration of basic PAS-calcium. Basic PAS-calcium is known to suppress pepsin action of gastric juice but such action is weak in Al-PAS-Ca. It has also been reported that Al-PAS-Ca gives hardly any side effects and its use over a long period is well tolerated.

4) G. Illari: Ann. Chim.(Rome), **42**, 32(1952)[C. A., **46**, 11153(1952)].
5) J. Gomi: Shinyaku-to-Rinsho (New Drugs and Clinics), **5**, 304(1956); H. Yamada: Sogo Rinsho (General Clinics), **5**, 194(1956); H. Yamada: Paper presented at the Kinki Local Meeting of the Japanese Society of Tuberculosis, October 29, 1955.

Experimental

Preparation of Al-PAS—A solution of 42 g. (0.2 mole) of PAS-sodium (dihydrate) and 8.4 g.(0.1 mole) of $NaHCO_3$ dissolved in 350 cc. of water was decolorized with activated carbon, filtered, and a solution of 24.1 g.(0.1 mole) of $AlCl_3 \cdot 6H_2O$ crystals dissolved in 300 cc. of water was added dropwise into this filtrate with stirring. After reacting for 5 hr. at room temperature, colorless precipitate formed, which was pasty at first but became crystalline. The precipitate was collected by suctional filtration, washed with water until Cl^- was no longer detected in the washing, and the precipitate was dried at 50° for 5 hr. Yield, 39.6 g.(94% of theory). Calcd. for $H_2\left[(C_7H_5O_3N)_2Al\begin{matrix}OH\\OH_2\end{matrix}\right] \cdot 3H_2O$: PAS content, 72.8; Al content, 6.42%. Found : PAS content, 72.93; Al content, 6.54%.

When dried on a porcelain plate, analytical values of the product gave PAS content of 65.91% and aluminum content of 6.08% (Calcd. : PAS content, 64.5; Al content, 5.7%), and agree with the hexahydrate.

Preparation of Al-PAS-Ca—To a stirred suspension of 21 g. of Al-PAS ($3H_2O$) in 100 cc. of water, ca. 6 g. of 20% ammonia water was added dropwise and the clear solution thus formed was stirred at room temperature while dropping 18.5 cc. of 40% $CaCl_2$ solution. The colorless crystalline powder that precipitated out was collected by filtration, washed with water, and dried at room temperature. Yield, 24.7 g.(93%). Calcd. for $Ca[(C_7H_5O_3N)_2Al(OH)(H_2O)] \cdot 7H_2O$: PAS content, 57.8; Al, 5.09, Ca, 7.55%. Found : PAS content, 56.9; Al, 5.00; Ca, 7.37%.

Determination of Al-PAS—About 400 mg. of Al-PAS was accurately weighed, placed in a 100-cc. measuring flask, 80 cc. of distilled water and 4 cc. of dil. HCl were added, and the crystals were dissolved with warming. After cool, this solution was diluted to 100 cc. with distilled water and this was used as the test solution.

(1) Determination of PAS : According to the method for determination of PAS-sodium in N.F. J. II.[6]

(2) Determination of Aluminum : (a) Twenty cc. of the test solution was measured accurately, 100 cc. of distilled water and 5 cc. of oxine solution (2 g. of oxine dissolved in 4~5 cc. of glacial AcOH with warming and diluted to 100 cc. with distilled water) were added, and the solution adjusted to pH 4.8~5.0 with ca. 20 cc. of 2*N* NaOAc solution (BCG pH-test paper used as an indicator). The solution was warmed at 50~60° for 30 min. and the precipitate formed was collected on a sintered glass filter by suctional filtration. The precipitate was washed with warm water (60°) until the filtrate became colorless.

The precipitate was dissolved with 25 cc. of 6*N* HCl, the filter was washed with water, and the acid solution and washings were placed in a 500-cc. measuring flask. The whole was brought to 500 cc. with water and 10 cc. of this solution was transferred to a 100-cc. measuring flask. After diluting this to 100 cc. with water, absorbancy (E) of this solution at 358 mμ was measured in 10-mm. cell and content of aluminum was calculated from the following formula :

$$\text{Al}\,(\%) = \frac{E_{358} \times 5}{0.1838 \times \text{amt.(mg.) of the subst. in 20 cc. of test solution}}$$

(b) The precipitate formed on addition of oxine and 2*N* NaOAc solution, with subsequent warming at 50~60°, in the above procedure (a) was collected by suctional filtration, washed with warm water (60°) until the filtrate became colorless, and the precipitate was transferred to an iodine bottle with the aid of 20 cc. of an equal-volume mixture of EtOH and $CHCl_3$. To this solution, 25 cc. of 0.1*N* Br_2 water was added, followed by 5 cc. of dil. HCl, stoppered immediately, and the bottle was shaken at 15° for 5 minutes. The mixture was allowed to stand at 15° for 30 min., 6 cc. of KI test solution was added cautiously, and the mixture was swirled gently. After 5 min., this solution was titrated with 0.1*N* $Na_2S_2O_3$, using 3 cc. of starch test solution as the indicator. A blank test was carried out at the same time. One cc. of 0.1*N* Br_2 water corresponds to 0.2248 mg. of Al.

Determination of Al-PAS-Ca—About 800 mg. of Al-PAS-Ca was weighed accurately, placed in a 200-cc. measuring flask, dissolved in 150 cc. of water and 4 cc. of dil. HCl, and the solution was brought to 200 cc. with water to be used as the test solution.

(1) Determination of PAS : Same as in Al-PAS described above.

(2) Determination of Aluminum : Same as in Al-PAS described above.

(3) Determination of Calcium : Accurately measured 100 cc. of the test solution was adjusted to pH 5~7 with addition of ammonia test solution, 10 cc. of ammonium oxalate test solution was added with stirring, and the mixture was heated on a water bath for 1 hr. The precipitate formed was collected by filtration, washed with hot water until the washing no longer became turbid within 1 min. after addition of $CaCl_2$, and the precipitate was washed into a beaker with 100 cc. of hot water,

6) National Formulary of Japan, Second Edition (English Edition), 408(1957). Japanese Ministry of Health and Welfare.

after punching a hole in the filter paper. The precipitate in the beaker was heated to 80° after addition of 30 cc. of dil. H_2SO_4 (1:3) and titrated with 0.1*N* $KMnO_4$. One cc. of 0.1*N* $KMnO_4$ corresponds to 2.004 mg. of Ca.

Conductometric Titration of Al-PAS·6H₂O—A solution of 0.104 g. of Al-PAS·$6H_2O$ dissolved in 30 cc. of dimethylformamide was titrated separately with *N* NH_4OH and *N* NaOH. In the former case, break at the equivalence point was not clear, while a white precipitate of aluminum hydroxide formed with the latter reagent, and titration was not effected.

Therefore, the usual measure taken in such a case was followed; as by partial titration with 0.1*N* NH_4OH solution and subsequent titration with 0.1*N* NaOH, thereby succeeding in the titration.

Conductivity curve is shown in Fig. 1 and the equivalence point calculated by the following formula agreed well with that calculated.

0.1*N* NaOH consumed (cc.) = 4.5 cc. × 1.001 = 4.5 cc.
Sample taken : Al-PAS·$6H_2O$ (mol. wt., 474) = 0.104 g.
Calcd. : 0.1*N* NaOH, 4.4 cc.

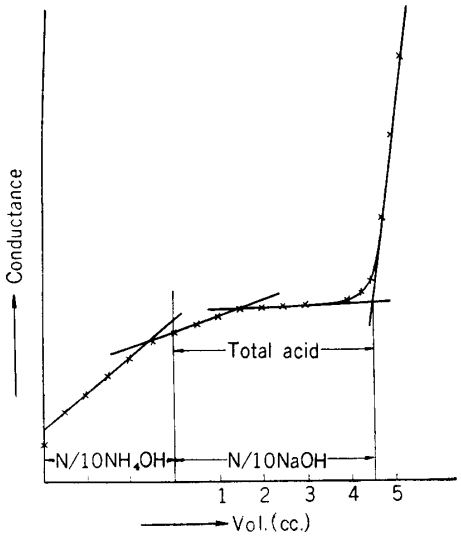


Fig. 1. Conductometric Titration of Al-PAS·$6H_2O$

0.1*N* NaOH (F = 1.001)
Solvent : 30 cc. of dimethylformamide
Sample taken : 104 mg.

This has shown that Al-PAS·$6H_2O$ is a dibasic acid and the values agree well with those calculated as a hexahydrate.

Summary

In order to improve some of the defects of PAS preparations now being used, such as the bad taste, inconvenience of administration, and occurrence of side effects, a new complex compound of PAS and aluminum, $H_2[(C_7H_5O_3N)_2Al(OH)(H_2O)]$ was synthesized by the application of 1 mole of aluminum chloride to 2 moles of PAS-sodium in aqueous solution, in the presence of 1 mole of sodium hydrogen carbonate. This substance was found to be a trihydrate or hexahydrate at room temperature.

This new complex, Al-PAS, is a dibasic acid and occurs as colorless, fine crystalline powder, without odor and slightly sweet to the taste, becoming astringent later, and dissolves in 2 moles of sodium hydroxide or ammonia water. This alumino-*p*-aminosalicylic acid was dissolved in 2 moles of ammonia water and 1 mole of calcium chloride was added, from which colorless and tasteless crystals were obtained. Its analytical values agreed with the formula of $Ca[(C_7H_5O_3N)_2Al(OH)(H_2O)]\cdot 7H_2O$.

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