Journal of the Malaysian Pharmaceutical Society, 6(3 & 4):33-43

# PANGAMIC ACID (VITAMIN B15)

## II. Structural Studies of Synthetic Preparations †

*By*

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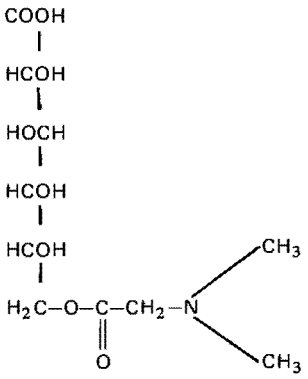
### INTRODUCTION

E.T. Krebs Sr. and his associates reported the discovery of pangamic acid in 1951 (Krebs *et al.*, 1951). Also named vitamin $B_{15}$ by these American investigators, this "new" water-soluble factor has been accorded little attention, especially by scientists in the "west". Little is known about it, while much controversies and contradictions exist. A review of the subject has been given in a separate article (Tee & Rasad, 1979).

The present report deals in detail with one particular aspect, namely, structural studies of pangamic acid. This report describes a study carried out to determine if the available synthetic preparations named "pangamic acid-vitamin $B_{15}$", or supposed to have the therapeutic properties of pangamic acid possess the required structure for the compound, namely 6-O-(N, N-dimethylglycyl)-D-gluconic acid:



*Structure of pangamic acid (vitamin $B_{15}$): (molecular formula: $C_{10}H_{19}O_8N$; molecular weight 281)*

### SYNTHETIC PREPARATIONS STUDIED

Six synthetic preparations called "pangamic acid-vitamin $B_{15}$", or supposed to have the therapeutic properties of this vitamin were studied:

1. Pangamic acid (vitamin $B_{15}$): purchased from Nutritional Biochemicals Division of ICN Pharmaceuticals Inc., Life Sciences Group, Cleveland, Ohio; referred to here as pangamic acid (ICN).
2. Calcium pangamate tablets from the Soviet Union, obtained through courtesy of Dr. J.C. Micheau, University of Toulouse, France; referred to here as calcium pangamate (USSR).
3. Calcium pangamate (D. Alam) refers to a product synthesised by an investigator in the University of Indonesia, Jakarta.
4. Gluconic$_{15}$ is marketed by Da Vinci Laboratories, Vermont, said to contain a mixture of calcium gluconate and dimethylglycine.
5. Liverall tablets are marketed by Sankyo, Japan, and said to contain a mixture of diisopropylammonium dichloroacetate and calcium gluconate.
6. Diisopropylammonium dichloroacetate (DADA) is also produced by Sankyo, Japan.

It must be noted that samples 4-6 are already considered not to be preparations of pangamic acid or vitamin $B_{15}$, since the manufacturers of these preparations have stated that the active component is not pangamic acid. They are however included in this study as they may be helpful in the identification of the other preparations studied.

### EXPERIMENTAL DETAILS

The preparations were subjected to a series of examinations, comprising of the following:

1. *Thin-layer chromatography.* Conditions and solvent systems used are as given in Figure 1 and Table I.

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2. *Reactions with some colour reagents.* Reaction with Folin and Ciocalteau (phenol) reagent was carried out according to two procedures: (a) that by Oser (1965); and (b) procedure described by Malakhova (1972). The ninhydrin test for free amino acids and the Benedict's test for reducing sugars were also carried out.

3. *Melting point determinations.* The Fisher-Johns Melting Point Apparatus was used.

4. *Nitrogen determination.* Nitrogen content of pangamic acid (ICN) was determined according to procedures described in Association of Official Analytical Chemists (AOAC, 1975).

5. *Spectroscopic studies.* Ultraviolet (UV) absorption spectroscopy was carried out using a Beckman DB-GT Grating Spectrophotometer. All UV spectra in this report have been obtained from 0.1% solutions of the samples in 0.01N HCl. Infra-red (IR) spectra were obtained using the potassium bromide disc procedure, scanned in a Perkin-Elmer Infra-red Spectrophotometer, model 700. IR spectrum for calcium pangamate (USSR) was carried out by the Chemistry Department, Petaling Jaya.

   Nuclear Magnetic Resonance (NMR) spectrum (sample in deuterium oxide) and Mass Spectrum(MS) of pangamic acid (ICN) were obtained through courtesy of the Chemistry Department, University Malaya, Kuala Lumpur.

6. *Ether extraction of the samples.* Samples 1, 4 and 5 were extracted with ether. After filtering and evaporating off the ether, the ether soluble fraction was obtained.

Both the ether soluble and insoluble fractions were studied by TLC, melting point determination and IR spectroscopy.

## RESULTS AND DISCUSSION

### 1. Thin-layer chromatography

A thin-layer chromatogram of the various preparations of "pangamic acid-vitamin $B_{15}$", together with dimethylglycine, calcium gluconate and other relevant compounds is shown in Figure 1. Table I shows the Rf value of pangamic acid (ICN) in all the four solvent systems used.

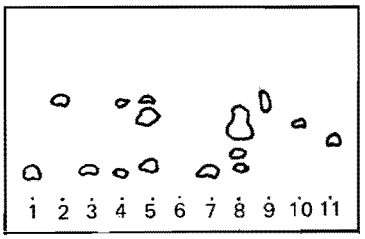



*Figure 1. Thin-layer Chromatogram of Various Synthetic Preparations of "Pangamic Acid-Vitamin $B_{15}$" (Developed with solvent system, as described by Telegdy-Kovats et al., 1969 & 1970).*

Layer silica gel G; Activation: 1 hour at 110°C; Solvent: n-propanol/ethyl acetate/25% ammonia/water (5:1:1:3): Distance travelled by solvent front 14 cm; Time of run: 2 hours. Spray reagent: alkaline potassium permanganate.

1 = Calcium gluconate
2 = Dimethylglycine hydrochloride
3 = Pangamic acid (ICN)
4 = Calcium pangamate (D. Alam)
5 = Calcium pangamate (USSR) (1 tablet in 50 ml 0.01N HCl)
6 = DADA - Sankyo
7 = Liverall (2 tablets in 50 ml 0.01N HCl)
8 = Gluconic – 15 (1 tablet in 50 ml 0.01N HCl)
9 = 6-O-(N,N-dimethylglycyl)-α-D-glucopyranose
10 = Glucose (0.1% in distilled water)
11 = Glycine (0.1% in distilled water)

(All solutions 0.1% in 0.01N HCl unless otherwise mentioned, 10 ul applied for all samples.)

Under the described conditions for TLC, pangamic acid (ICN) appears to give a single spot in all four solvent systems. It is further noted that this spot has the same mobility as calcium gluconate. This would tend to indicate that the spot given by this preparation from ICN may actually be a gluconate or gluconic acid. It is not very probable that calcium gluconate and pangamic acid should migrate

**Table I**

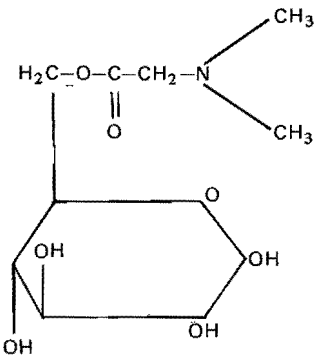
**Rf Values (x 100) of Pangamic Acid (ICN) in Thin-layer Chromatograms Developed in Various Solvent Systems**

| *Solvent System* | *Pangamic Acid (ICN)* | *Pangamic Gluconate* | *Dimethyl-glycine-HCl* |
|---|---|---|---|
| 1. n-propanol/ethyl acetate/ 25% $NH_3$/ water (5:1:1:3, v/v) | 17 | 18 | 39 |
| 2. n-butanol/acetic acid/ water (2:1:1, v/v) | 31 | 33 | 24 |
| 3. n-propanol/25% $NH_3$/ water (6:2:1,-v/v) | 13 | 13 | 45 |
| 4. Chloroform/acetic acid/ water (3:3.5:0.5, v/v) | 16 | 17 | 24 |

*Note:* For all chromatograms, silica gel C layers, activated for 1 hour at 110° C was being used.

together in all these solvent systems studied. This represents the first finding that points to the possibility of the absence of pangamic acid from ICN preparation.

Further support to this suggestion is seen from the migration of 6-O-(N,N-dimethylglycyl) -α-D-glucopyranose (synthesised by the laboratory of Dr. J.C. Micheau, University of Toulouse, France; structure of the compound is as given below). This compound, structurally very much related to pangamic acid, migrates very close to dimethylglycine, rather than to calcium gluconate.




*Structure of 6-O-(N,N-dimethylglycyl)-α-D-glucopyranose*

From Figure 1, it is seen that all the preparations studied, except for sample 6, contain a few components. Calcium gluconate and dimethylglycine are seen in most of them, and glucose in some of the preparations. The presence of glycine is not clearly shown in the chromatogram.

Using the mobility of 6-O-(N, N-dimethylglycycl)-α-D-gluco-pyranose in the chromatogram as a guide, one may say that the presence of pangamic acid in all the preparations studied is not clearly indicated since dimethylglycine hydrochloride also migrates with the same Rf.

**2. Reactions with some colour reagents**

Results of reactions of the various samples with the three colour reagents are summarised in Table II.

All the preparations studied are negative to ninhydrin. This is in agreement with results obtained by TLC that glycine may not be present in the samples. Benedict's test for glucose and reducing sugars again give similar results as TLC.

It has been found that free dimethylglycine gives an intense blue colour with the Folin Ciocalteau (phenol) reagent and this is taken as a positive result. 6-O-(N, N-dimethylglycycl)-α-D glucopyranose, with a bound dimethylglycine group, however does not react with the reagent. This appears to suggest that the reagent reacts only with free dimethylglycine. As such, the reagent cannot be considered to be suitable for the detection of pangamic acid, contrary to the report of Malakhova (1972).

Hence, those samples given in Table II that react with the phenol reagent contain free dimethylglycine. Results from colour reactions are generally in agreement and support findings from TLC. The spot in calcium pangamate (D. Alam) and calcium pangamate (USSR), migrating close to 6-O-(N,N-dimethylglycyl)-α-D-glucopyranose and dimethylglycine in the TLC may really be dimethylglycine.

**Table II**

**Reactions of Various Synthetic Preparations of Vitamin $B_{15}$ with Some Colour Reagents**

| *Sample* | *Colour Reagents* | | |
|---|---|---|---|
| | *Folin & ciocalteau* | *Ninhydrin* | *Benedict's* |
| 1. Pangamic acid (ICN) | – | – | – |
| 2. Calcium pangamate (USSR) | + | – | + |
| 3. Calcium pangamate (D. Alam) | + | – | – |
| 4. Gluconic-15 | + | – | + |
| 5. Liverall | – | – | + |
| 6. DADA - Sankyo | – | – | – |
| 7. Capsule W/P | – | – | + |
| 8. Glucose | – | – | + |
| 9. Gluconolactone (Merck) | – | – | – |
| 10. Glucono-δ-lactone (Wako) | – | – | + |
| 11. Calcium gluconate | – | – | – |
| 12. Dimethylglycine hydrochloride | + | – | – |
| 13. 6-O-(N, N-dimethylglycycl)-α-D-glucopyranose | – | – | + |
| 14. Glycine | – | + | – |

*Note:* Samples 8-14 are not supposed to be preparations of "Vitamin $B_{15}$", but relevant compounds included to aid in study.

### 3. Melting point characteristics

Observations made during melting point determinations of the various "vitamin $B_{15}$" preparations are summarised in Table III.

The label on pangamic acid (ICN) states that the preparation has a melting point (with decomposition) at 160–170°C. The sample indeed decomposed at this temperature range. However, it is also found that there is another component which melts at 110–120°C. This indicates that the preparation contains another component, although TLC, for reasons that will be discussed below, only shows a single spot. It is also noted that this latter temperature range is the same as the melting point of diisopropylammonium dichloroacetate, as seen from DADA from Sankyo.

All the preparations studied, except for DADA, decomposed at 160–170°C, which is also the temperature of decomposition of calcium gluconate. Most of them also show a broad melting point range, indicating that they are mixtures of compounds. These findings are in agreement with those from TLC.

**Table III**

**Melting and Decomposing Characteristics of Various Synthetic Preparations of "Vitamin $B_{15}$ – Pangamic Acid"**

| *Sample* | *Observations* |
|---|---|
| 1. Pangamic acid (ICN) | Melting of some crystals at 110–120° C. remaining (majority) decompose (with charring or browning) and melt at 160–170°C. |
| 2. Calcium pangamate (USSR) | Charring starts at 160°C, continues, with melting of crystals up to 200°C. |
| 3. Calcium pangamate (D. Alam) | Charring of crystals begins at 160° C, continue to darken, and melt up to 200 C. |
| 4. Gluconic 15 | Melting at 120°C and charring at 160–170°C. |
| 5. Liverall | Appearing of very tiny droplets of liquid at 115°C. Charring and melting at some crystals at 160° C, spreading slowly till 185° C, when rapid charring and melting occurs until 200° C. |
| 6. DADA – Sankyo | Melting at 105–115° C. |
| 7. Glucose | Melting at 145° C, no charring observed when heated up to 200° C. |
| 8. Calcium gluconate | Melting with charring at 160–170° C. |
| 9. Gluconolactone. | Melting at 150° C, heating up to 200°C, no charring observed. |
| 10. Dimethylglycine hydrochloride | Melting at 170–180° C. |
| 11. 6-O-(N,N-dimethyl-glycyl)-α-D-gluco-pyranose | Melting with charring at 170–180° C. |

*Note:* Samples 7–11 are not supposed to be preparations of "Vitamin $B_{15}$" but are relevant compounds included to aid in this study.

### 4. Nitrogen content determination

Eight separate determinations of pangamic acid (ICN) for its nitrogen content gives an averaged value of 3.25% with a range of 2.81 to 3.87%. According to the molecular formula of $C_{10}H_{19}O_8N$ for pangamic acid, it has been calculated that the nitrogen content required is 4.98%. The obtained result therefore gives a rather large deviation (of about 35%) from the calculated value. It may therefore be said that either the preparation is impure, or that it has a different structure and formula from that of pangamic acid.

### 5. Spectroscopic studies

Ultra-violet absorption spectra of pangamic acid (ICN) and calcium pangamate (D. Alam) are given in Figures 2 and 3. These spectra are far from similar to that for 6-O-(N,N-dimethylglycyl)- α - D-glucopyranose, the analogue of pangamic acid (see Figure 4). On the other hand, they are similar to the spectra given by DADA, calcium gluconate and Liverall (Figures 5,

6 and 7). This therefore indicates that the two preparations in question may be similar in structure to gluconate and diisopropylammonium dichloroacetate on the one part, and dissimilar to 6-O-(N,N-dimethylglycyl)-α-D-glucopyranose on the other part. It is difficult to draw any conclusions from the UV spectrum of calcium pangamate (USSR) in Figure 8 as it is a mixture of at least 3 compounds basing on Figure 1.

Infra-red spectra for pangamic acid (ICN) and calcium pangamate (USSR) are given in Figures 9 and 10. It is noted that the spectra lack an absorption band at 1740 $cm^{-1}$. However, if one looks at the structure of panga-

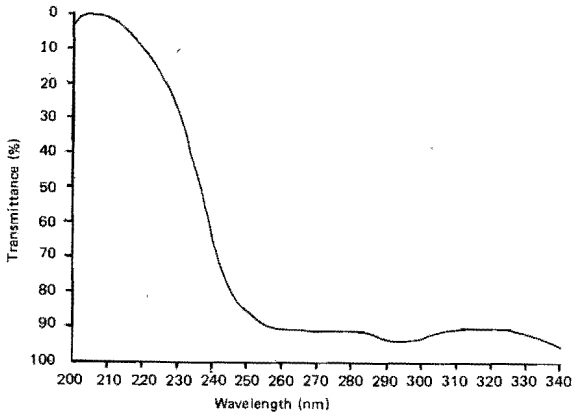



*Figure 2. Ultra-violet Absorption Spectrum of Pangamic Acid (ICN)*

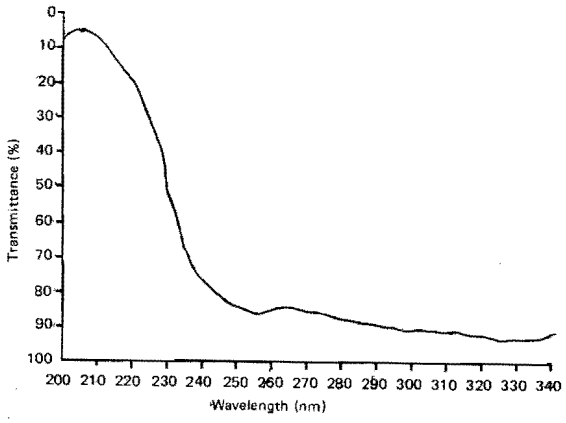



*Figure 3. Ultra-Violet Absorption Spectrum of Calcium Pangamate (D. Alam)*

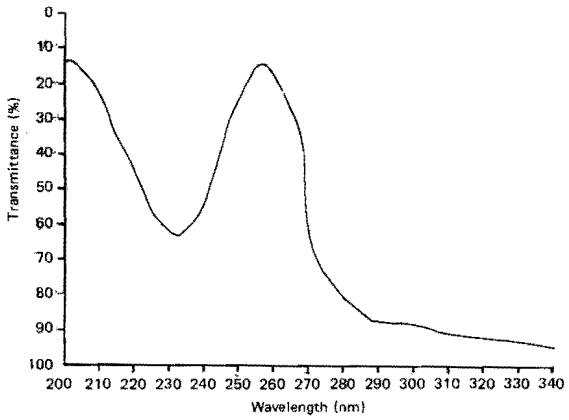



*Figure 4. Ultra-violet Absorption Spectrum of 6-O-(N,N-Dimethylglycyl)-α-D-Glucopyranose*

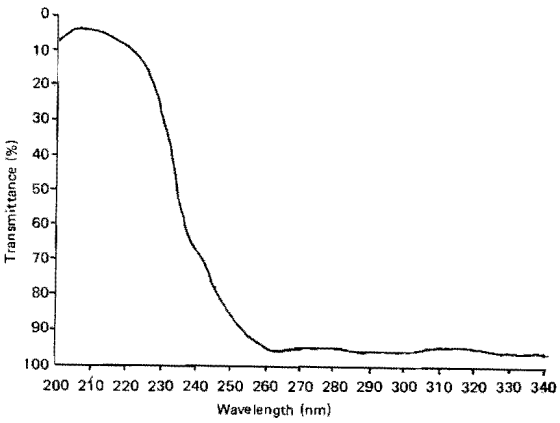



*Figure 5. Ultra-Violet Absorption Spectrum of DADA-Sankyo*

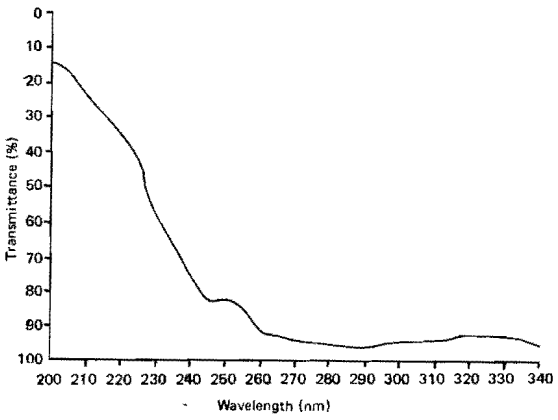



*Figure 6. Ultra-Violet Absorption Spectrum of Calcium Gluconate*

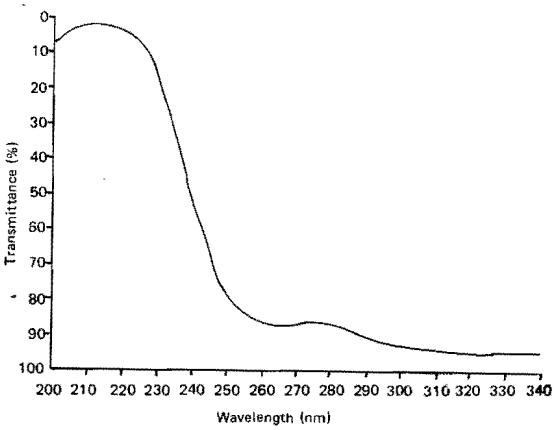



*Figure 7. Ultra-Violet Absorption Spectrum of Liverell*

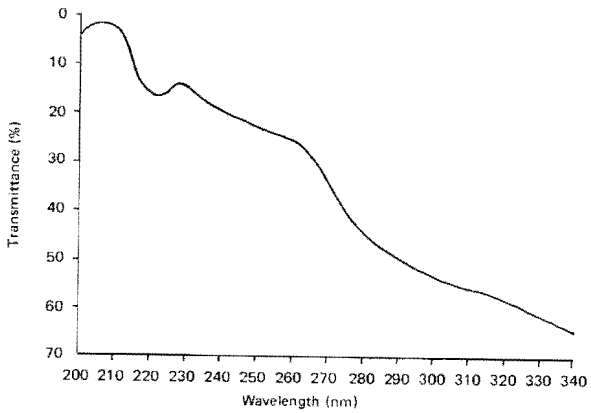
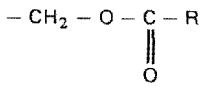



*Figure 8. Ultra-Violet Absorption Spectrum of Calcium Pangamate (USSR)*

mic acid, it is clear that the compound is an ester of the acetate type, with a carbonyl ester group:



which should give an absorption band at 1740 cm $^{-1}$. It must therefore be said that these two preparations do not have the required structure for pangamic acid.

To support this, the IR spectrum of 6-O-(N,N-dimethylglycyl)-α-D-glucopyranose was obtained, and given in Figure 11.

The carbony, ester group present in this analogue of pangamic acid is clearly shown at 1740 cm $^{-1}$ in the spectrum.

It is of importance to mention that all the IR spectra of samples of "pangamic acid" reported in the literature (Janicki *et al.*, 1968; Telegdy-Kovats *et al.*, 1970 and 1976) do not have a distinct peak at 1740 cm$^{-1}$.

Mass spectrum of this preparation from ICN Pharmaceuticals (Figure 12) shows that the highest mass recorded is 234.

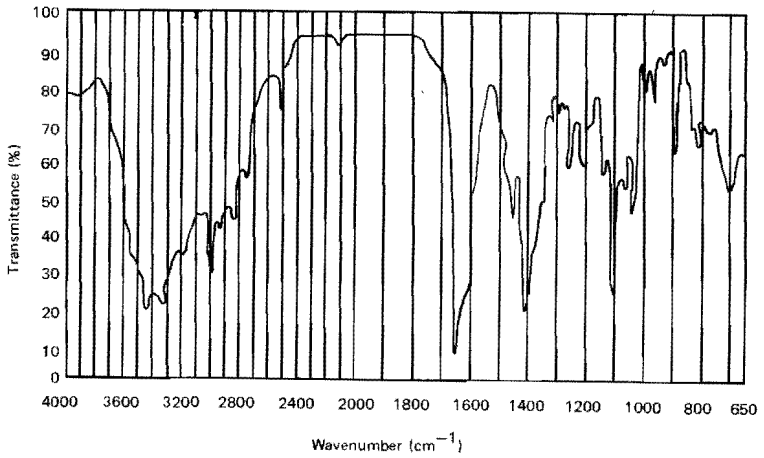



*Figure 9. Infra-Red Spectrum of Pangamic Acid (ICN) in Potassium Bromide Disc*

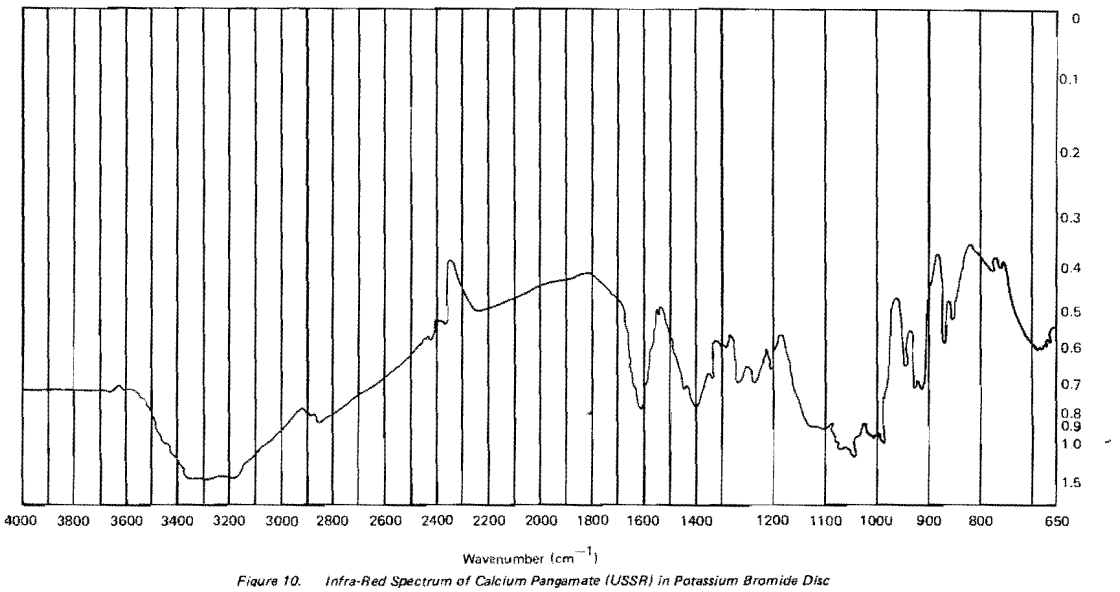



*Figure 10. Infra-Red Spectrum of Calcium Pangamate (USSR) in Potassium Bromide Disc*

The molecular weight indicated therefore is not consistent with the molecular weight for pangamic acid, which is 281. The NMR spectrum (Figure 13) shows that the structure of the preparation studied is not in agreement with the required structure for pangamic acid. This spectrum disagrees with the proton chemical shift data reported in the literature for pangamic acid (Lacan *et al.*, 1971), and with the data given by Micheau (1970) for the NMR spectrum for 6-O-(N, N-dimethylglycyl)-α-D-glucopyranose. The MS and NMR spectra have therefore provided further proof that the preparation in question could not have the structure of pangamic acid.

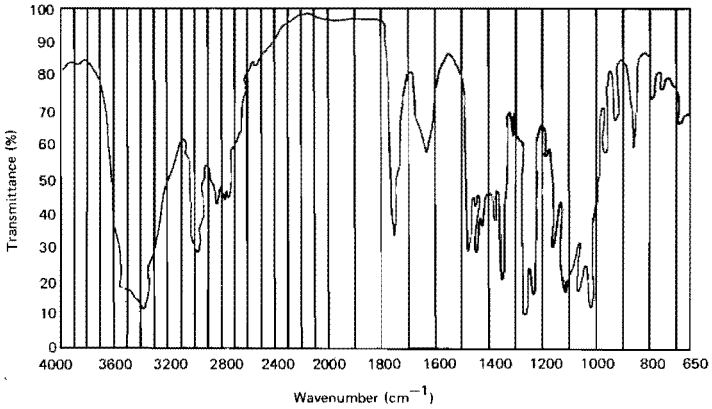



*Figure 11. Infra-Red Spectrum of 6-O-(N,N-Dimethylglycyl)-α-D-Glucopyranose in Potassium Bromide Disc.*

6. **Ether extraction of the preparations.**

(a) *Pangamic acid (ICN)*: It has been found that this preparation gives an ether soluble fraction when extracted with the solvent. This should not be so if the preparation is a pure, authentic sample of pangamic acid, said to be a water-soluble vitamin. Melting point determination of the ether soluble fraction gives a temperature of 115°C, similar to that of DADA from Sankyo. The ether insoluble fraction, on the other hand, has been found to be decomposed at 190°C, resembling calcium gluconate.

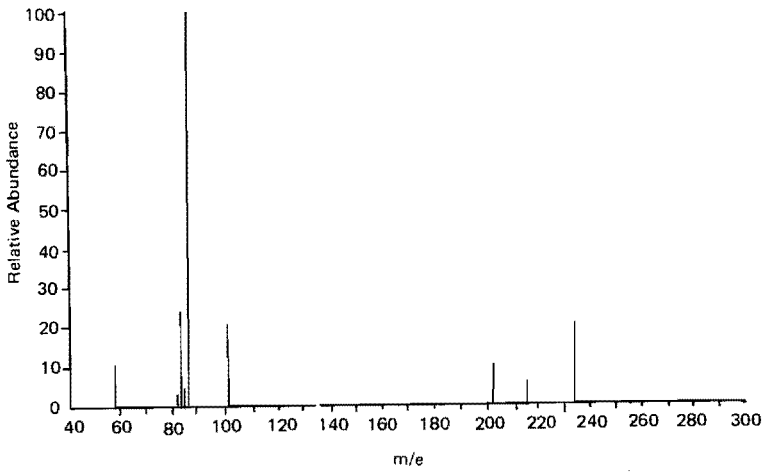



*Figure 12. Mass Spectrum of Pangamic Acid (ICN)*

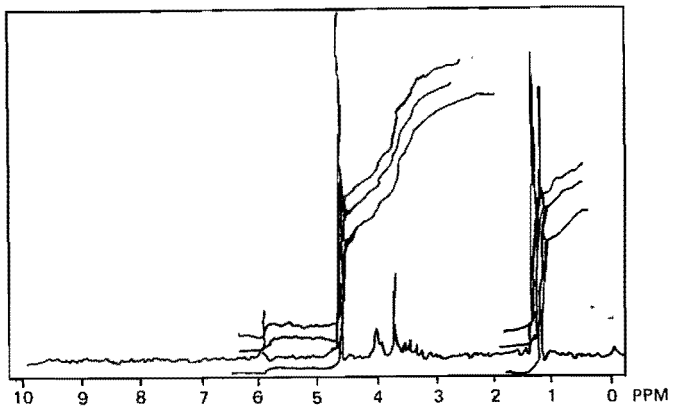



*Figure 13. Nuclear Magnetic Resonance Spectrum of Pangamic Acid (ICN) in Deuterium Oxide*

Both these fractions from ether extraction were next chromatographed on thin-layer plates, together with DADA-Sankyo and gluconate. Using the solvent system of butanol/acetic acid/water (described earlier) it has been shown that the ether insoluble fraction has the same Rf as calcium gluconate. The ether soluble fraction, on the other hand, migrates as DADA-Sankyo. This lends support to the earlier findings that pangamic acid (ICN) may actually contain two components, i.e. a gluconate and diisopropylammonium dichloroacetate.

It is noted that earlier TLC has shown that only one spot is given by pangamic acid (ICN) and no spot at all for DADA-Sankyo (Figure 1). It is believed that this is because the spray

reagent, potassium permanganate, is not sufficiently specific for diisopropylammonium dichloroacetate. Later chromatography of DADA-Sankyo and the ether soluble fraction was carried out using very high concentrations, so that a faint spot was detected.

For the final proof of findings from melting point studies and TLC of the fractions from ether treatment, IR spectroscopy was used. Crystals of the fraction soluble in ether, when scanned in the spectrophotometer gives the spectrum as shown in Figure 14. DADA-Sankyo gives an IR spectrum, shown in Figure 15. Comparing these two spectra, there is no doubt at all that the ether soluble fraction from pangamic acid (ICN) is indeed diisopropylammonium dichloroacetate; both spectra are identical in every single absorption peak obtained.

(b) *Liverall:* A parallel study done on this preparation gives similar findings. The ether soluble fraction again gives a melting point of 115°C, while the ether insoluble fraction has a melting point (with decomposition) at 190°C. This again shows the presence of diisopropylammonium dichloroacetate and calcium gluconate in the sample. In fact, Liverall has been stated by the manufacturer as containing these two compounds.

(c) *Calcium pangamate (USSR):* Ether extraction of this preparation does not give any crystals upon evaporating off the ether. It is therefore suggested that the sample does not contain diisopropylammonium dichloroacetate, in agreement with TLC and melting point studies carried out on the sample prior to ether treatment.

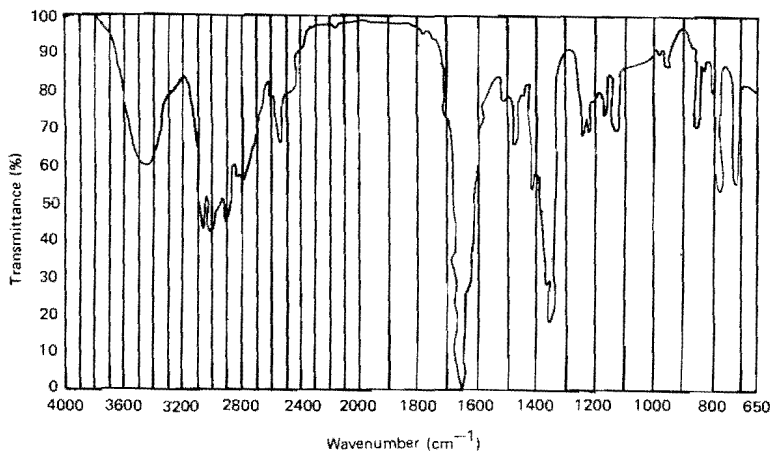



*Figure 14. Infra-Red Spectrum of DADA-Sankyo in Potassium Bromide Disc*

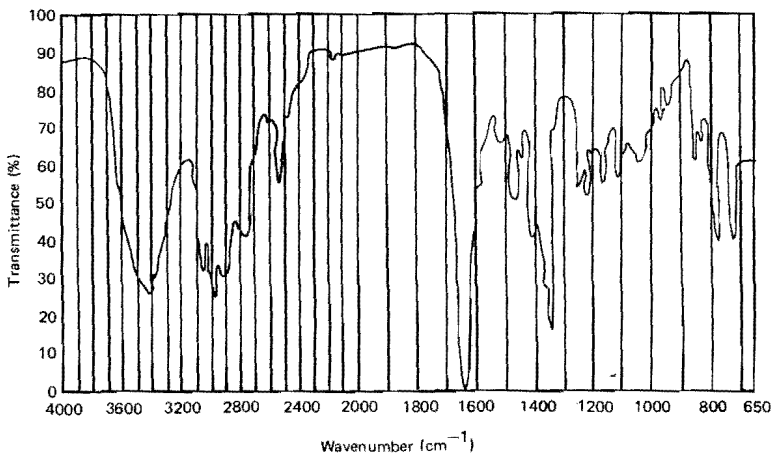



*Figure 15. Infra-Red Spectrum of Ether Soluble Fraction of Pangamic Acid (ICN) in Potassium Bromide Disc*

## CONCLUSION

From the various studies carried out, it must be concluded that none of the synthetic preparations called "pangamic acid-vitamin $B_{15}$", or supposed to have the therapeutic properties of pangamic acid, may be said to contain pangamic acid. Other investigators, for example Casu *et al.* (1958), French & Levi (1966) and Micheau *et al.* (1972) had also pointed out that synthetic preparations of the "vitamin" may not have the required structure. This appearance of numerous preparations, loosely called "pangamic acid" or "vitamin $B_{15}$", but really do not contain 6-O-(N,N-dimethylglycyl-D-gluconic acid is probably due to the misconception among some investigators on the actual structure of pangamic acid. It must be noted that this has important implications. It is clear that if such preparations are used for physio-pharmacological studies and the results obtained attributed to that of pangamic acid or vitamin $B_{15}$, this can be misleading and confusing. The reported biochemical and therapeutic properties of pangamic acid must be taken as tentative until it has been ascertained that the reports had been studied using authentic preparations of pangamic acid.

## SUMMARY

Stemming from reports in the literature that synthetic preparations called "pangamic acid-vitamin $B_{15}$" may not contain the required structure for pangamic acid, i.e. 6-O-(N,N-dimethylglycyl)-D-gluconic acid and the disagreement among investigators on the structure of this so-called vitamin $B_{15}$, it has been felt important to study some such preparations. A series of examinations were carried out on these preparations, including one from ICN Pharmaceuticals, Cleveland, Ohio. Through parallel studies using an analogue of pangamic acid, namely 6-O-(N,N-dimethylglycyl)-α-D-glucopyranose, these examinations, consisting of thin-layer chromatography, colour reactions, melting point and nitrogen content determinations, and spectroscopic studies, have shown that none of the preparations studied may be considered to contain pangamic acid. They are really mixtures of two or more of the following: diisopropylammonium dichloroacetate, gluconate, glucose and dimethyl glycine. The implications of the availability of such preparations, loosely called "pangamic acid-vitamin $B_{15}$" are pointed out.



## ACKNOWLEDGEMENTS

*The authors express their gratitude to the Board of Directors and Staff of the SEAMEO Regional Graduate Applied Nutrition Course, Jakarta, for providing the facilities and funds to carry out this study. They would like to take this opportunity to thank the Director, Institute for Medical Research, Kuala Lumpur, for his kind permission to Tee E Siong to participate in the M.Sc. A. Nutr. Course. Their sincere thanks also go to all who had given, or helped to obtain the samples of "vitamin $B_{15}$" studied here, especially to Professor B.A. Underwood, Penn. State University, USA, and Dr. J.C. Micheau, University of Toulouse, France. Grateful thanks also to drs. Edwin, Jakarta Municipal Health Office, and Mr. Sartono and Mr. Chusosi of the Technical School of the Pharmaceutical Chemistry Laboratory, Jakarta, for the loan of their UV and IR Spectrophotometers.*


## SOME CLARIFICATIONS ON NOMENCLATURE.

In view of the confusion over the chemical identity of pangamic acid, it seems important to state correctly the chemical names of the compounds under discussion and to point out those which are vague and incorrect.

1. The following correct chemical names should be used:

For pangamic acid:

6-O-(N, N-dimethylglycyl)-D–gluconic acid *or*
6-O-(dimethylaminoacetate)-D-gluconic acid *or*
6 -O-(dimethylaminoacetyl)-D-gluconic acid.

For vitamin $B_{15}H_8$:

6-O- { bis { bis{1-methylethyl}amino acetatel }-D-gluconic acid *or*
6-O- bis{diisopropylamino)acetyl}-D -gluconic acid.

2. The following vague and/or incorrect names appearing in some publications should be avoided:

For pangamic acid:

(i) gluconodimethyl aminoacetate
(ii) D-gluconodimethyl aminoacetate *and*
(iii) glucono-6-acetyl-N-dimethylamine

For vitamin $B_{15}H_8$:

(i) gluconic acid 6-bis (diisopropylamino) acetate *and*
(ii) gluconodi - (diisopropylamine) acetate.

3. 6-O-(N, N-dimethylglycyl-α-D-glucopyranose is also known as:

6-O- (dimethylaminoaceate)- α -D-glucopyranose, *or*
6-O-(dimethylaminoacetyl)-α-D-glucopyranose.

4. Owing to the confused state of affairs on pangamic acid, entries of different Chemical Abstracts Registry Numbers were made, as shown in the following:

304-56-3 refer to
6-O-{bis (dimethylamino) acetate}-D-gluconic acid (pangamic acid)

11006-56-7 refer to
pangamic acid without a listed chemical name

20858-86-0 refer to
6-O-(N, N-dimethylglycyl)-D-gluconic acid (pangamic acid)

13149-69-4 refer to
6-O- { bis { bis(1-methylethyl}amino acetate } -D-gluconic acid (pangamic acid)

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However, the present paper defines pangamic acid as 6-O-(N, N-dimethylglycyl)-D-gluconic acid with 20858-86-0 as the Chemical Abstracts Registry Number.


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