On EUCALYPTUS KINOS, their VALUE for TINCTURES, and the NON-GELATINIZATION of the PRODUCT of CERTAIN SPECIES.

By Henry G. Smith, f.c.s., Assistant Curator, Technological Museum, Sydney.

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The greatest objection to the kino of *Pterocarpus marsupium* is the tendency it has to gelatinize when made into tinctures. Many methods have been advanced at one time or another to overcome this difficulty, but in most cases with doubtful success, as at present more than one Pharmaceutical Association is requesting a formula for tincture of kino that will keep. From the results of this investigation it appears that the best way to overcome this difficulty is to discard *Pterocarpus* kino altogether, and to *use those Eucalyptus kinos that do not gelatinize in tinctures.* Pharmacists need not be troubled with gelatinized tincture of kino no matter how long it is kept.

In the Pharmaceutical Journal (1841-2, p. 399) there is a paper by Mr. Redwood which is well worth reading by those interested in this matter. He there discusses the formation and constitution of this gelatinous substance, and calls attention to the statement by Dr. Thomson that the product of *Eucalyptus resinifera*[1] has the property of forming a tincture which gelatinizes on keeping. Dr. Pereira also states that when gelatinized tincture of kino occurs, that probably the Botany Bay kino (inspissated juice of *Eucalyptus resinifera*) had been employed (4th Edition,

[1] *Eucalyptus resinifera*, as we know it to day, has practically a Stringy Bark, and the "Ironbark" kinos (as *E. siderophloia*) consist largely of *Emphloin* and do not dissolve in alcohol.

Vol. II., p. 327). I have mentioned these statements to indicate how easily a whole group of substances may be condemned on the unsatisfactory behaviour of perhaps but one member. What the species of Eucalyptus was from which that kino was obtained it is not now possible to say. The present day *E. resinifera* only exudes kino in very small amount, so that it could not be collected in sufficient quantity to be of any use commercially, even if the kino was of use for tincture, which it is not. It is probable that the "Botany Bay kino" above referred to was the product of several species of Eucalyptus collected indiscriminately, and owing to the facility with which it gelatinized in tinctures, it probably contained a predominance of kinos obtained from such species as *E. pilularis*, *E. piperita*, *E. hæmastoma*, etc., trees which were at that time plentifully distributed throughout the vicinity of Botany Bay. That this is so, is shown from the description given by Dr. Pereira[1] of the kino from Botany Bay that he had met with, which was in irregular odourless masses, many of which were in the form of tears. Some were almost black, and when digested in water, swelled and became soft and gelatinous, like red currant jelly. The kino also acted similarly when digested in rectified spirit. There seems little doubt from the above that that particular sample of kino was of considerable age, and that it was obtained from species allied to the "Stringybarks," or the "Peppermints," because with age all this class of kinos become almost black, and are then but little soluble in either water or alcohol, but swell up like a jelly. The sample could not have been obtained from *E. siderophloia*, which species has been thought to have been the original *E. resinifera*, because the solubility of the "Ironbark" kinos in water is but little impaired by age.

[1] Pereira, Mat. Med. 4th Ed., Vol. II., pp. 237-8 (1857).

On the other hand the "New Holland kino" mentioned by Dr. Thomson[1] as being procured by wounding the *Eucalyptus resinifera*, gave a brown chocolate powder on which cold water acted but slowly, but boiling water formed a deep cherry-red solution which threw down a brick coloured precipitate on cooling. The solution was coloured *deep green* by sesquichloride of iron. From these reactions it is certain that that sample of kino was not obtained from any species belonging to the "Ironbarks," or the "Stringybarks," or the "Peppermints." The above statements denote clearly that no care was taken to distinguish between products of separate species.

At the International Exhibition of 1862 a collection of vegetable products was exhibited from Tasmania, and in a series of notes on these by Mr. W. Archer, F.L.S., published in the Technologist, appears the following:—"This gum, which seems to have similar properties to those of the East Indian kino, exudes from woods of all the Tasmanian species of Eucalyptus." That these exudations were at that time, collected without discrimination, is indicated by the above statements.

The evidence which will be submitted later will illustrate how unsatisfactory these Eucalyptus kinos must be when so collected, and it also offers an explanation for the non-agreement in the experiences of various writers, who have described their successes or otherwise with particular methods suggested at various times for the preparation of tincture of kino.

In the journals devoted to pharmacy much information may be found dealing with this subject. It has been supposed that glycerol had the desired effect of preventing gelatinization, and its addition is of course official, but R.

[1] Thomson, Mat. Med. p. 678 (1843).

Rother[1] considers glycerol "as unsatisfactory as all other agents previously tried. The alleged occasional success with sundry corrigents can only be accounted for by the fact that there are numerous varieties of kino, and that one or more of these may not be susceptible of this change." It is very probable that this is so, and it is remarkable that while some Eucalyptus kinos gelatinize very readily, others do not do so even after the lapse of many years.

About seven years ago I made tinctures (1 in 10) of half a dozen Eucalyptus kinos, but omitted the glycerol. These were put up in glass stoppered bottles and kept in the dark continuously. The kinos of *E. amygdalina*, *E. macrorrhyncha* and *E. piperita* readily gelatinized and formed a perfect jelly after a comparatively short time. The kino of *E. corymbosa* has become thick but not even now a jelly, that of *E. punctata* has slightly thickened, but the kino of *E. calophylla* has undergone no alteration after all these years. The evidence thus obtained has been followed up with gratifying results.

The questions naturally arising are, why this variability in gelatinization when the tinctures are made under identical conditions, and what is the cause of the gelatinization? These will be considered together. It may perhaps be generally accepted that the cause of the gelatinization is the same in those Eucalyptus kinos which gelatinize and in the kino of *Pterocarpus marsupium*.

Pereira considered the jelly to consist principally of pectin and tannic acid (page 238) and Dorvault that it was pectic acid, but Mr. Redwood (*loc. cit.*) after experiment, arrived at the conclusion that neither pectin nor pectic acid was present in the jelly, but thought that the change was traceable to "ulmic acid" or "humus."

[1] American Journal of Pharmacy, 1886, and Pharm. Journ., July 1886, p. 67.

Mr. J. H. Hustwick[1] points out that with one sample of kino he obtained gelatinized and non-gelatinized tinctures by different methods of working. In one case he treated the granular kino with the alcohol without powdering, and consequently an insoluble portion was left which was discarded; this tincture kept well and was as fluid after two years as when made. In the other the kino was powdered and this tincture readily gelatinized. I have referred to this peculiar experience because it bears directly upon what will be shown later.

Mr. Rother (*loc. cit.*) suggests the addition of catechu to the tincture to prevent gelatinization, but from my experiments the addition of a moderate amount of a non-gelatinizable kino to a gelatinizable one does not prevent the ultimate production of a jelly, although it retards it considerably, and in direct ratio to the amount of the former kino or tincture added. (See Table III.)

Mr. G. W. Kennedy[2] advocates the addition of logwood, but it is probable that this acts in the same way as does catechu, and I do not think it would be finally successful unless it was added in large amount.

Mr. G. M. Beringer[3] describes a method for making the tincture with diluted alcohol, but this apparent improvement was probably due to most of the active gelatinizing principle in the kino being left behind in the "dregs" on the filter. Nor will the freshness of the kino help permanently although of some advantage, but fresh or aged the cause of the gelatinization appears to be present in the kino, and will naturally do its work in time. So it is with the Eucalyptus kinos that gelatinize, and Mr. Maiden's qualification[4] for Eucalyptus kinos that will make satis-

[1] Pharm. Journ. [3] 2. 260.
[2] American Journ. of Pharm., Feb. 1880.
[3] American Journ. of Pharm., 1903, p. 378.
[4] Pharm. Journ., Oct. 1889, p. 323.

factory tinctures will unfortunately not now hold, because those Eucalyptus kinos which are, when fresh, completely soluble in cold water as well as in alcohol, are those that gelatinize the most readily. These rapidly gelatinizable kinos are numerous, and are obtained from Eucalypts whose oils contain the terpene phellandrene, such species are *E. pilularis*, *E. piperita*, *E. amygdalina*, (and its allies), *E. hæmastoma*, *E. obliqua*, *E. macrorrhyncha*, *E. Sieberiana*, *E. oreades*, etc. It is a pity that this gelatinization takes place so readily, because these soluble kinos are the most astringent, and when quite fresh are almost as astringent as gallo-tannic acid itself, but owing to the objectionable property of gelatinizing, the whole of the members of this class of Eucalyptus kinos will have to be discarded for tincture making. It should be possible, however, to use them in other directions, but the method by which this could be done is a matter for future determination. Fortunately there are several Eucalyptus kinos which do not gelatinize in tinctures, and these are almost equally astringent with those kinos mentioned above and are readily obtainable.

The kinos of the Eucalypts contain at least three tannins determinable by reagents; two of these gelatinize in tinctures, the other does not. Of the two gelatinizable tannins the one which gives the violet colour and precipitate with ferric chloride gelatinizes much more readily than the one which gives a green colour with ferric chloride.[1] It is possible that the tannins in these kinos may be separated, perhaps through their copper salts.

It was the somewhat regular increment of particular oil constituents in progressive species that enabled the new

[1] The late Mr. Henry Trimble (the author of "The Tannins") expressed an opinion to the author of this paper, that eventually a new tannin might be isolated from the Eucalypts, and the results here recorded support strongly that supposition.

substances to be most easily isolated. The chemistry of the kinos appears to run in a parallel direction, so that it is possible by following down the species, to find a kino in which a required tannin occurs in a maximum amount.

As two only of these kino tannins appear to gelatinize in tinctures, the cause of the gelatinization may perhaps be indicated. The latest suggestion is that advanced by Mr. E. White[1] where he endeavours to show that the formation of gelatinized tincture of kino is due to the presence of an enzyme. Whether this is so can only be completely solved by its isolation. If an enzyme is the cause then it seems strange that it only acts on certain of these kino tannins, if an oxydase then the product of its action does not form a solid compound with one at least of these tannins. That some action of this sort does take place is suggested by the rapidity with which some of the tannins in Eucalyptus kinos may be made to gelatinize by the addition of a very small amount of formaldehyde, and in a lesser degree by acetaldehyde. The rapidity of the gelatinization brought about by the addition of a few drops of formaldehyde, or of acetaldehyde, can be seen from the tabulated results (Table I.) These results are comparable with those obtained with the tinctures tested by age alone in the ordinary way. The test seems to be a reliable one and by its aid it appears possible to determine whether a kino will gelatinize in tinctures or not. No doubt the test is a severe one and will detect all kinos that might possibly gelatinize.

Although it may not be easy to isolate the unorganised ferment, if such is present, yet, it is not difficult to isolate and grow the organised substance which appears to be present in most Eucalyptus kinos. This substance is easily obtained from those kinos which gelatinize the most readily. It always grows at the bottom of the diluted aqueous kino

[1] Pharm. Journ., May 1903, p. 644, and Nov. 1903, p. 702.

solution and appears to start from the particles of kino which are always left undissolved. Mr. S. J. Johnston, B.A., B.Sc., of the Technological Museum is making an investigation of this organism, and its description will appear later.

The following list gives the general chemical reactions and astringency values of the Eucalyptus kinos dealt with in this paper, and it will be seen that associated kinos behave similarly. In no instance did the time allowed for precipitates to form extend beyond one hour. The method of determining the astringency values was described in the previous paper on the kino glucoside, the strength of the solution being one gram per litre.

The method adopted for taking the colour reaction with ferric chloride was to have a full test tube, standing before a window, and to allow one drop of the reagent to fall through the solution without agitation; the strength of the kino solution for this test was 0·33 gram per litre. The best results were obtained with a solution of this strength. The other tests were made with kino solutions of one gram per litre.

Most Eucalyptus kinos contain mixed tannins and the reactions are, therefore, largely governed by the predominant tannin present. The tannins which give violet and green colorations with ferric chloride gelatinize in tinctures, the former much the more readily, but the one giving the blue coloration does not gelatinize. It is also possible to detect the diminution or otherwise of the individual tannins by the reaction with ferric chloride and by the astringency value and the gelatinization test. The changes that take place in the colour reaction, together with the results of other reagents are not given in the table, as they have little bearing on the results of gelatinization. Those Eucalyptus kinos that give a blue coloration with the ferric salt, a sparce precipitate slow to form with iodine in potassium iodide, and a comparatively small amount of the copper salt insoluble in ammonia, all contain in excess the tannin which does not gelatinize in tinctures.

	Astringency Value.	Coloration at once with Ferric chloride.	Iodine in Potassium Iodide.	Bromine water.	Cupric sulphate and ammonia in excess.	Zinc acetate.
E. Sieberiana ...	777	violet	dense ppt. soon forming	ppt. at once	dense flocculent ppt.	ppt at once
E. dives	777	ditto	ditto	ditto	ditto	ditto
E. pilularis ...	838	ditto	ditto	ditto	ditto	ditto
E. corymbosa ...	694	green	ppt. soon forms	ditto	not a large ppt.	ditto
E. trachyphloia ...	729	greenish purple	ditto	ditto	considerable ppt.	ditto
E. rostrata ...	541	green	precipit. slowly forms	ditto	finely divided precipitate	ditto
E. rostrata, Bosisto, Victoria	576	green	ditto	ditto	ditto	ditto
E. goniocalyx ...	435	green	ditto	ditto	not large ppt.	ditto
E. Woollsiana ...	235	reddish-brown	ditto	ditto	very slight ppt.	turbidity
E. melliodora ...	247	bright-green	ppt. very slow to form	ditto	no ppt.	ditto
E. populifolia ...	518	purplish grey	ppt. slow to form	ditto	very slight ppt.	ppt. at once
E. Bridgesiana ...	412	greenish grey	ditto	ditto	slight ppt.	ditto
E. hemiphloia ...	200	reddish-brown	ditto	ditto	very slight ppt.	turbidity
E. pendula ...	482	purplish grey	ditto	ditto	fair ppt.	ppt. at once
E. Smithii ...	565	bluish-green	ditto	ditto	slight ppt.	ditto
E. Dawsoni ...	541	purplish grey	ditto	slight ppt.	considerable ppt.	ditto
E. intertexta ...	518	brownish grey	ppt. very slow to form	ditto	ditto	ditto
E. oleosa	717	violet	considerable ppt. soon forms	ppt. at once	ditto	ditto
E. calophylla 1897	753	blue	ppt. very slowly forms	ppt. soon forms	slight ppt.	ditto
E. calophylla 1895	729	ditto	ditto	ditto	ditto	ditto
E. eximia... ...	435	ditto	ditto	ditto	ditto	ditto
E. microcorys ...	741	ditto	no ppt.	no ppt.	ditto	ditto
E. maculata ...	612	ditto	turbidity	slight ppt.	small ppt.	ditto
Gallo-tannic acid	1000	ditto	no ppt.	no ppt.	considerable ppt.	ditto

From the following tables which deal with the results of the gelatinization of the tinctures, it is apparent that some species of Eucalyptus give kinos of great excellence for tincture making. They do not gelatinize even after many years, and the addition of glycerol is not needed. The probable gelatinization of all kinos can also readily be determined by simple tests. The kinos here listed were chosen as being representative of the whole 100 species of Eucalyptus examined. The remainder, not here enumerated, gave the chemical reactions agreeing with these, but so far as this investigation has gone no others were detected giving indications of non-gelatinizable kinos. The list of these is thus restricted to the four following species :—

1. "Tallowwood," *Eucalyptus microcorys*, Eastern Australia.
2. "Red Gum," *Eucalyptus calophylla*, Western Australia.
3. "Mountain Bloodwood," *Eucalyptus eximia*, Blue Mountains, N. S. Wales.
4. "Spotted Gum," *Eucalyptus maculata*, Eastern Australia.

The kinos of *E. eximia* and *E. maculata* (being closely related chemically) give precipitates when diluted with water, which peculiarity might be an objection to their use pharmaceutically. The tincture of *E. calophylla* gives only a slight turbidity when diluted with water, while the tincture of *E. microcorys* remains perfectly clear and transparent on the addition of water.

There seems but little to choose between the kinos of *E. microcorys* and *E. calophylla* for tincture making. The latter, however, can be readily obtained in any quantity. They do not undergo change when kept in the dry state. There seems no reason, apparently, why the kinos of some Eucalyptus species should not eventually replace, for

medicinal and official purposes, all other kinos from whatever source obtainable.

TABLE I.

This table shows the comparative rates of gelatinization of tinctures (1 in 10) of the following Eucalyptus kinos with

(*a*) Formaldehyde—commercial formalin of which 20 drops from the same pipette contained ·191 gram HCHO.

(*b*) Acetaldehyde, of which 20 drops contained ·091 gram CH_3CHO.

Experiments were commenced 14th January 1904, when 5 drops of each aldehyde were added to 5 cc. of separate tinctures of each kino, agitated, and stood on one side for 20 hours.

After that time, to those tinctures which had not solidified, 5 drops more of each aldehyde were added to the respective tinctures, and these again stood for 20 hours.

To those tinctures which had not solidified, 5 drops more of aldehyde were again added to the respective tinctures, and observation repeatedly made until 1st July, 1904. 5 cc. of tincture were taken in all cases. Those tinctures which slowly solidified became much thicker previous to forming a jelly. In the table the following may also be noted:—

(*a*) *E. pilularis* being a quite fresh kino did not so readily gelatinize with acetaldehyde as did the kino of *E. dives*.

(*b*) *E. pilularis* after boiling acted similarly to the unboiled kino.

(c) *E. Woollsiana*, *E. hemiphloia*, *E. pendula*, and *E. intertexta* did not form homogenous jellies with formaldehyde, but a precipitate separated.

(*d*) *E. rostrata* and *E. calophylla* (2 samples of each) show that the kinos from identical species of Eucalyptus act similarly irrespective of location or date, again illustrating the chemical constancy of Eucalyptus species.

	Formalin 5 drops	Formalin 10 drops	Formalin 15 drops	Acetaldehyde 15 drops
E. Sieberiana, collected May 1899	15/1/04 brown jelly formed	...	...	2/2/04 jelly formed
E. dives, Oct. 1898	,,	...	...	2/3/04 jelly formed
E. pilularis, Jan. 1904	,,	...	...	1/7/04 very thick but not a solid jelly
E. corymbosa, April 1889	15/1/04 no change	16/1/04 light brown jelly form ed	...	1/7/04 had thickened considerably
E. trachyphloia	,,	16/1/04 no change	18/1/04 plum coloured jelly formed	1/7/04 thickened a little
E. rostrata, March 1903	,,	,,	18/1/04 dark salmon coloured jelly formed	1/7/04 still fluid
E. rostrata, Bosisto, Victoria	,,	,,	,,	,,
E. goniocalyx, April 1889	,,	,,	21/1/04 salmon coloured jelly formed	,,
E. Woollsiana, April 1901	,,	,,	10/2/04 partly solid and partly fluid	1/7/04 a homogenous salmon color'd jelly formed
E. melliodora, March 1903	,,	,,	25/1/04 salmon coloured jelly formed	1/7/04 still fluid
E. populifolia, Nov. 1899	,,	,,	18/1/04 salmon coloured jelly formed	,,
E. Bridgesiana, March 1899	,,	,,	22/1/04 salmon coloured jelly formed	1/7/04 thickened a little
E. hemiphloia, Dec. 1900	,,	,,	25/4/04 partly solid and partly fluid	1/7/04 still fluid
E. pendula, Dec. 1900	,,	,,	,,	,,
E. Smithii, Oct. 1900	,,	,,	22/1/04 salmon coloured jelly formed	,,

	Formalin 5 drops	Formalin 10 drops	Formalin 15 drops	Acetaldehyde 15 drops
E. Dawsoni, Oct. 1893	15/1/04 no change	16/1/04 no change	18/1/04 salmon coloured jelly formed	1/7/04 thickened a little
E. intertexta, May 1903	,,	,,	25/4/04 partly solid and partly fluid	1/7/04 partly solid and partly fluid
E. oleosa, May 1903	,,	16/1/04 light brown jelly formed	...	1/7/04 had thickened considerably
E. calophylla, June 1897	,,	16/1/04 no change	1/7/04 as fluid as when put up	1/7/04 as fluid as when put up
E. calophylla, Dec. 1895	,,	,,	1/7/04 no change	1/7/04 no change
E. eximia, Jan. 1898	,,	,,	,,	,,
E. microcorys, April 1896	,,	,,	,,	,,
E. maculata, July 1898	,,	,,	,,	,,
E. pilularis, boiled kino.	15/1/04 brown jelly formed	...	...	1/7/04 thickened but not yet a jelly
Angophora intermedia April 1888	15/1/04 no change	16/1/04 no change	19/1/04 dark brown jelly formed	20/1/04 dark brown jelly formed
Pterocarpus marsupium India	,,	16/1/04 brown jelly formed	...	2/3/04 quite thick 25/4/04 brown jelly formed
Eucalyptus paniculata March 1904	A tincture (1 in 10) was made of this kino by dissolving in a small quantity of water, adding twice the amount of 90% alcohol, and filtering from the small quantity of bark. To 5 cc. of tincture 5 drops of formalin were added. Next day a perfect jelly had formed. The tannin in the "Ironbarks" is thus again shown to be allied to that of the "Peppermints" as *E. dives*, etc.			

Table II.

This table shows the influence a slowly gelatinizable kino, like that of *Eucalyptus Woollsiana*, has upon a rapid gelatinizable one, like that of *Eucalyptus dives*. The mixtures were prepared on the 21st January 1904, and five drops of commercial formalin added to each.

	22/1/04	23/1/04	25/1/04	26/1/04	22/2/04
0·5 cc. of *E. Woollsiana* 4·5 cc. of *E. dives*	slight jelly	solid jelly formed	...	...	purplish-brown jelly
1 cc. of *E. Woollsiana* 4 cc. of *E. dives*	thickened a little	solid jelly formed	...	...	brown jelly
2 cc. of *E. Woollsiana* 3 cc. of *E. dives*	no change	slight jelly	solid jelly formed	...	jelly becoming salmon coloured
3 cc. of *E. Woollsiana* 2 cc. of *E. dives*	...	no change	thickened a little	solid jelly formed	dark salmon coloured jelly
4 cc. of *E. Woollsiana* 1 cc. of *E. dives*	...	...	no change	thickened a little	salmon coloured jelly

NOTE.—The predominant colours of these jellies are those of the jellies of the individual kinos themselves, showing that these colours are not accidental.

TABLE III.

This table shows the preventative action a non-gelatinizable kino like that of *Eucalyptus calophylla*, has when mixed with a gelatinizable one, like that of *Eucalyptus pilularis*. Tinctures of the two kinos were combined in the proportions stated, and five drops of formaldehyde (commercial formalin) added to each mixture. The mixtures were prepared on the 11th February, 1904.

	12/2/04	13/2/04	15/2/04	17/2/04	18/2/04	25/4/04
0·5 cc. of *E. calophylla* 4·5 cc. of *E. pilularis*	thicken'd a little	solid jelly formed				
1 cc. of *E. calophylla* 4 cc. of *E. pilularis*	no change	solid jelly formed				
2 cc. of *E. calophylla* 3 cc. of *E. pilularis*	...	thickened a little	solid jelly formed			
3 cc. of *E. calophylla* 2 cc. of *E. pilularis*	...	no change	no change	slight jelly	solid jelly formed	
4 cc. of *E. calophylla* 1 cc. of *E. pilularis*	...	...	...	no change	no change	slight jelly, solid on April 30