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ISOLATION AND CONSTITUTION OF THE NARCOTIC SUBSTANCE FROM KAWA-KAWA, (PIPER METHYSTICUM).

BY

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The method of isolation, properties and structure of the narcotic substance from kawa-kawa *(P. methysticum)*, are described. This substance occurs in a concentration of 1 % in the dried plant material (old stems and roots) and is the actual active substance in this material. The active substance, marindinin, is easily obtained in a crystalline condition by a combination of an appropriate method of extraction with adsorption analysis. The ultimate purification is much more troublesome. Finally, marindinin appeared to be identical with the dihydrokawain of Borsche, who did not recognise this substance as the active principle, however, which he was able to isolate only with difficulty and in very small yield from the kawa resin. Attention is drawn to the fact that marindinin must be brought into the form of a fine emulsion before it can exert its narcotic qualities.

In a preliminary publication [1]) we reported briefly on the isolation of the narcotic substance from kawa-kawa or wati, which had not previously been carried out sucessfully. In a second publication [2]) we gave a literature survey of the many investigations, which have been carried out so far on this interesting plant and the substances it contains. We also included in this article a description of the phenomena, which make their appearance when men make use of this narcotic (in this case, the Marindinese in South New Guinea).

We showed that there are varieties of kawa with a high activity and some with low activity. The usual mastication of roots and stems and consumption of the expressed liquid does not cause, as is frequently supposed, a fermentation of the material by the enzymes in the saliva resulting in an activation of the active substance, but an intense emulsification, by which the active subtsance is more

[1]) Proc. Akad. Wetenschappen Amsterdam **41**, 855 (1938).

[2]) Geneeskundig Tijdschr. Nederland. Indië **78**, 1941 (1938).

completely and rapidly resorbed and consequently the narcotic action shows itself more decidedly. Also the difference between the activity of the tonic-acting so called "kawa-tea" and that of the masticated material can be satisfactorily explained in this way. Finally we described in detail the beautiful work of W. Borsche on the various substances in kawa; he did not succeed, however, in isolating the active (narcotic) substance [3]).

The present communication deals with the method of isolation and the investigation of the active substance which we have called *marindinin*.

We used both dried material (old stems), which we received from Merauke (New Guinea) and fresh stems from plants planted by ourselves and which we dried in the sun. In this process the weight diminished to one fourth to one sixth. In our attempts to isolate the active substance we took our lead from animal experiments (monkeys and pigeons); the reader is referred to the previous publications. The substance to be examined was always converted into a finely divided aqueous emulsion either with a little cocoanut oil and lecithin, or gum arabic or saliva.

It soon appeared, taking Borsche's work into account, that in all probability only adsorption analysis could lead to the desired result.

The dried and finely milled material, generally in portions of about 300 g, was extracted intensively in a Soxhlet apparatus with light petroleum (b.p. 60—80°) for a few hours until the extract was practically colourless. If the material was extracted with ether, more extract was obtained but it was less active and frequently dark coloured. On extraction with light petroleum (provided it was not too prolonged) a pale yellow extract is obtained from which a large amount of crystalline matter (methysticin, yangonin, etc.) soon separated; this material was inactive in animal tests. The clear petroleum-ether filtrates usually contained only 8—12 g of non-crystallisable material, which was subsequently purified by adsorption analysis. The most suitable adsorbent appeared to be well dried "acid clay", a product produced in Java; various sorts of alumina, franconite etc. were not suitable.

The extract is allowed to run slowly through a column of acid clay 25 × 3.3 cm and is subsequently developed with light petroleum

[3]) For a literature survey, as well as for photographs of the kawa plant and microphotographs of the active substance we isolated, we would refer the reader to our second communication, in which the technique for the animal experiments (with monkeys and pigeons) is briefly described.

containing respectively 5, 10 and 15 % of anhydrous, peroxide-free ether. In this way there is produced a broad green band (sometimes green with a very small red and yellow ring on top), which passes through the column relatively quickly, while another very broad green band remains behind at the top of the column. After the bands have been separated as much as possible by development, the column is cut up into various fractions and each eluated separately with cold alcohol. The middle eluates are pale green, the outermost deep green. The distribution of the adsorbed material over the whole column is fairly uniform; the eluate from the uppermost green band crystallises well but is inactive in animal tests. The eluate from the lowest green ring does not crystallise and is also inactive. On the other hand, eluates from the colourless part of the column are all active, and more active as they are derived from lower parts of the column. When the active alcoholic eluates are evaporated in a vacuum, the lowest eluates (above the green band) crystallise almost completely, the others do not crystallise or crystallise only partially. The latter can be purified further, however, by a second adsorption analysis (a small amount of the eluate from the lowest green band dissolved in petroleum ether being added as an "indicator") and induced to crystallise better. By repeated adsorption, however, the activity gradually diminishes.

The crystals so obtained are much more difficult to bring into the form of an emulsion (for the animal experiment) than the amorphous fractions of the original extracts. There were no indications, that any other active fractions besides these crude crystallates were present in our light petroleum extracts. The average weight of crude, active, crystalline material is about 3—4 g from 300 g of dried stems. The substance may be further purified by dissolving in ether, adding petroleum ether and allowing the solution to evaporate spontaneously in the air. In this way large colourless prisms (microphoto loc. cit.) are obtained, which dissolve with more difficultly in ether as they become purer. These crystals are very active; the mother liquors are inactive. It is perhaps superfluous to remark that we satisfied ourselves that the phenomena produced in our animal experiments by the crystalline material were identical with those produced by the crude extracts and the crude starting material. 50—70 mg sufficed for a pigeon; about 500 mg for a monkey weighing a kilo; one must take into account, however, that the latter animals resist the oncoming tendency to sleep, which makes it necessary to give relatively large doses.

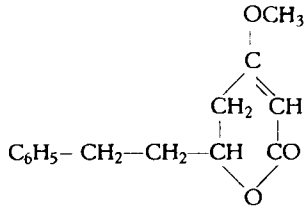
We were also able to isolate these crystals from the "Resina

Kawa" of the firm of Riedel de Häen (see previous publication).

The crystals melt at 60° and have a specific rotation of +30°; we called this substance marindinin (l.c.). In this attempts to establish the constitution of this substance it appeared that it could not be homogeneous. Therefore we tried to purify it further by recrystallisation from ether, and we succeeded only after repeated recrystallisation. While the elementary analysis of the impure marindinine indicated a composition $C_{15}H_{18}O_3$, the purer product (*i. e.* recrystallised until the analytical figures no longer altered) had the composition $C_{14}H_{16}O_3$. The activity in animal tests remained practically unchanged, while the material from the mother liquors was only slightly active. The melting point, crystalline form and optical rotation were unaltered; from this it appears that the crude marindinin must be contaminated with an impurity which is difficult to remove and which has similar properties, thus probably a higher homologue. This could not be obtained in a crystalline condition.

Pure marindinin is a lactone, which gives an isomeric acid $C_{14}H_{16}O_3$, m.p. 133°, after hydrolysis with strong alkali. On heating with dilute sulphuric acid, this acid is split up into methyl alcohol and carbon dioxide and an α-β-unsaturated methyl ketone, $C_{12}H_{14}O$; hence the starting product was the methylated enol form of a β-ketonic acid with 13 carbon atoms.

The ketone just mentioned was hydrogenated catalytically to the saturated ketone $C_{12}H_{16}O$. This substance gave a crystalline semicarbazone, which could also be made synthetically, and was converted by oxidation with hypobromite into ω-phenylvaleric acid, the amide of which was identical with the analogous synthetic product. Thus the structure was completely elucidated, viz.



From its physical and chemical properties, degradation products etc., this substance is identical with the dihydrokawain of Borsche[4]). That the latter did not recognise dihydrokawain as

[4]) Alas according to his communication Prof. Borsche had none of the substances he isolated from kawa available for comparison tests, so that we were obliged to carry out the whole series of degradation reactions.

the active constituent of kawa, in spite of the fact that he isolated it, is possibly to be ascribed to the method of testing with animals, on which no further data are given. He obtained dihydrokawain by hydrogenating kawain, and it can also be isolated with considerable trouble from kawa resin itself [5]). Since marindinin is responsible for the most striking pharmacological properties of kawa and since it differs so markedly from all the other kawa-substances, we propose to retain this name in place of the less characteristic "dihydrokawain".

That the least unsaturated of all the kawa-substances shows the strongest pharmacological action, may perhaps depend on the fact that the other substances (*e.g.* methysticin and yangonin) are difficult to emulsify, or it may be that they are more quickly oxidised in the organism on account of their relatively strongly unsaturated character.

EXPERIMENTAL PART.

Extraction of the active substance.

320 g of dried and milled old stems, from material from our own plantation (corresponding with 1.8 kg of fresh material), were extracted in a Soxhlet apparatus for 4 hours with boiling petroleum ether (b.p. 60—80°); the solvent was renewed after every hour. The four, pale yellow, turbid extracts were combined and after some time a large amount of crystalline and oily materials separated. The latter was carefully brought into solution by adding a little ether to the supernatant petroleum ether solution and shaking vigorously. This treatment was continued until the oil had dissolved. The weight of crystalline material was then 4.3 g, the volume of the extract containing about 5 % of ether was 1000 cm^3 and it contained about 7.5 g of dry residue.

Adsorption analysis.

The solution obtained above is allowed to run through a column of acid clay measuring 25 × 3.3 cm. Within 24—48 hours the extract filters through of its own accord: a green ring about 4 cm broad is formed at the top of the column, a second band about 3 cm broad is formed half way up. The bands are now developed, first with a mixture of light petroleum and 10 % of ether and then with one containing 15 % of ether. After about 400 cm^3 have been used the lower band is at the bottom of the column. The latter is now divided into five parts, the first and the fifth corresponding with the uppermost and the lower green bands respectively. The second, third and fourth fractions are about equal in size; these are eluated separately several times with 95 % alcohol and the alcoholic eluates

[5]) Borsche, Peitzsch, Ber. **58**, 2414 (1930).

evaporated to dryness in a vacuum. The second fraction remains oily (0.50 g), the third crystallises to a large extent (0.65 g) and the fourth fraction crystallises almost completely (0.70 g).

According to tests on pigeons with 50 and 100 mg of substance, respectively, (the material being first of all emulsified to give a fine emulsion with gum arabic and hot water), the second fraction is weakly active and the other two strongly active.

Subsequently the second fraction is combined with similar fractions from other experiments and again chromatographed when it affords crystals identical with those from the third and fourth fractions.

When older stems are taken as the starting material, the combined weight of the active fractions may amount to 3.5—4 g.

Isolation.

The two last mentioned crystallisates are dissolved in ether and carefully precipitated with light petroleum. First of all a little amorphous, sticky material separates, and then marindinin in large, transparent prisms, m.p. 60°, $[\alpha]_D = +30°$, insoluble in light petroleum and water, moderately soluble in ether, readily soluble in alcohol and chloroform. The product is repeatedly recrystallised from ether with a little light petroleum; the above physical constants and the activity in animal tests do not alter but the percentage composition does. At last this becomes constant. The substance reacts neutral in alcoholic solution, dissolves in boiling caustic alkali solution; after acidification, another substance with acid properties separates. This substance is not stable in contact with hot mineral acids but gives carbon dioxide and is converted into a pleasant smelling oil.

The impure substance with constant properties gave:

3.850 and 4.590 mg gave 2.450, 2.980 mg H_2O and 10.320, 12.290 mg CO_2.
Found H = 7.12, 7.2; C = 73.10, 73.00 %.

Pure substance with constant properties and composition gave:

1.260 mg of substanee in 23.750 mg of camphor gave $\triangle = 10.3°$.
6.940 mg of substance used 2.8 cm³ 0.01 n. $AgNO_3$ (Zeisel).
3.839, 4.201 mg of substance gave 2.37, 2.59 mg H_2O; 10.19, 11.13 mg CO_2.
6.930 mg on hydrolysis consumed 1.60 cm³ 0.02 n NaOH.
Found H = 6.91, 6.90; C = 72.39, 72.26; M.W. = 236.
OCH_3 = 12.5 %; lactone number = 217.
Calc. for $C_{15}H_{18}O_3$, H = 7.3, C = 73.2, M.W. = 246.
$C_{14}H_{16}O_3$, H = 6.8, C = 72.4, M.W. = 232, OCH_3 = 13.7 %.

Degradation.

Acid. 1.0 g of marindinin is boiled with 20 cm³ of 10 % sodium hydroxide solution for a few minutes until everything has dissolved to a clear solution. After cooling the mixture is acidified with sulphuric acid and extracted with ether. On evaporation of the

ethereal solution, 0.9 g of a well crystallised, colourless substance with acid properties and a melting point of 130°, is obtained. The melting point can be raised to 133° (corr.) by recrystallisation from methyl alcohol. The substance is not optically active.

2.930 mg of substance gave 2.47 mg H_2O and 10.44 mg CO_2.
13.43 mg consumed 3.00 cm^3 0.02 n. alkali.
7.740 mg consumed 3.20 cm^3 0.01 n. $AgNO_3$ (Zeisel).
Found H = 7.02, C = 72.28, OCH_3 = 12.8 %: equiv. 225.
Calc. for $C_{14}H_{16}O_3$. see above.

When crude marindinin having the composition $C_{15}H_{18}O_3$, is hydrolysed, a mixture of acids with a much lower melting point is produced and this mixture is difficult to purify. This indicates the heterogenity of the crude material.

Ketone. 0.85 g of the acid was dissolved in a little ether and then boiled with 5 cm^3 of 5 % sulphuric acid. On evaporation of the ether an oil was obtained, which rapidly split off carbon dioxide. This was taken up in ether and shaken with soda. After drying with sodium sulphate and evaporation of the ether, a yellow, pleasant smelling, neutral oil with ketonic properties was obtained. This substance decolorised potassium permanganate rapidly, added bromine immediately, resinified with caustic alkali and no longer contained methoxyl. The oil was hydrogenated in 10 cm^3 of ethyl acetate with 15 mg of platinum oxide, when 1 mol. of hydrogen (H_2) was taken up. The hydrogenated product gave a well defined semicarbazone, m.p. 143°, which gave no melting point depression with the semicarbazone of ω-phenyl-butyl methyl ketone.

3.155 mg gave 0.508 cm^3 N_2 (28°/759 mm): N = 18.23 %.
Calc. for $C_{13}H_{19}ON_3$, N = 18.03 %.

The semicarbazone was split by boiling 600 mg with 7 cm^3 of 10 % oxalic acid solution for eight hours and then extracting with ether. 0.5 g of saturated ketone was obtained, which was shaken for 10 hours with hypobromite solution from 5 g of caustic soda, 5 g of bromine, and 50 cm^3 of water. In this way an oily acid was produced, which was very stable and distilled at 150° in a high vacuum. The acid amide, m.p. 107°, gave no depression of the melting point when mixed with the amide of ω-phenylvaleric acid, which melts at the same temperature.

3.810 mg gave 2.930 mg H_2O, 10.410 mg CO_2.
Found H = 8.6, C = 74.52 %.
Calc. for $C_{11}H_{15}ON$, H = 8.5, C = 74.6 %.

Batavia (Java), *Chemical Section of the Eijkman-Institute (Medical Laboratory)*, February 1939.

(Received February 23rd 1939).