

CANTHARIDIN FROM MYLABRIS PUSTULATA Fb., INDIA.

By B. H. Iyer and P. C. Guha.

Cantharidin is a colourless, crystalline substance much used as a vesicant, and is contained in more than a dozen medicinal preparations for internal and external use. Owing to its high blistering power internal administration requires great caution, as it irritates the alimentary canal and causes marked vasoconstriction; a cantharidin-content of 1 in 100,000 is the prescribed internal dose. Cantharidin is used in tuberculosis, lupus, cystitis, incontinence of urine and many other diseases. It is used externally in the form of plasters and tincture more than internally, and is also used in very minute doses as a hair-tonic, cantharidin hair-oil being an example.

Cantharidin is extracted from a type of insect commonly known as blister beetles, formerly used as such, or as an oily extract for blistering. R. Hughes Buller's observation in this connection is worth mentioning: "In India these beetles are used in a somewhat similar manner. In Baluchistan, for instance, the 'Gojak' beetle (closely allied to *Mylabris pustulata*) is soaked in milk and the fluid poured on the bald spots to cure mange." (From *Indian Insect Pests*, by H. Maxwell-Lefroy).

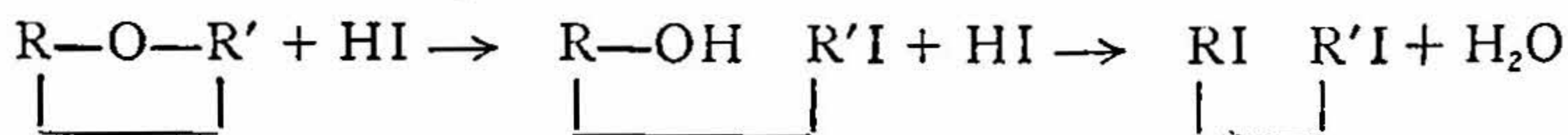
The blister beetles belong to the order *Coleoptera*, the two important species of this order which yield cantharidin being *Cantharis vesicatoria*, or Spanish Fly and *Mylabris pustulata*. The former are widely distributed in southern Europe and are quite different in appearance from *Mylabris pustulata*, being longer and having green upper wings. In Europe they are the chief source of cantharidin, but owing to the large variety of insects found in the tropics, the exact nomenclature and species of the family used for this work could not be identified with certainty from the available literature. Specimens were therefore sent to the Government Entomologist, Coimbatore, and afterwards to the Imperial Bureau of Entomology, London, for confirmation, when the insects proved to be *Mylabris pustulata* Fb., India.

At present, the dried insects, or extracted cantharidin preparations are imported at high cost, and an ample supply of blister beetles being available locally, the opportunity was taken to ascertain the cantharidin-content of these. Inquiry into the possibility of rearing the insects as a source of cantharidin has shown that their life-history is not yet

sufficiently well known to offer a prospect of success. The Government Entomologist, Coimbatore, informs us that the triangulin, or first stage grub, feeds upon grasshopper eggs, and this requirement suggests that it would be more profitable to collect the adult beetles at those periods when thousands infest the blossoms and tender shoots of numerous plants. Moreover, their removal for cantharidin-extraction would help to diminish their destructive activity, which in some districts is a serious menace to crops and gardens.

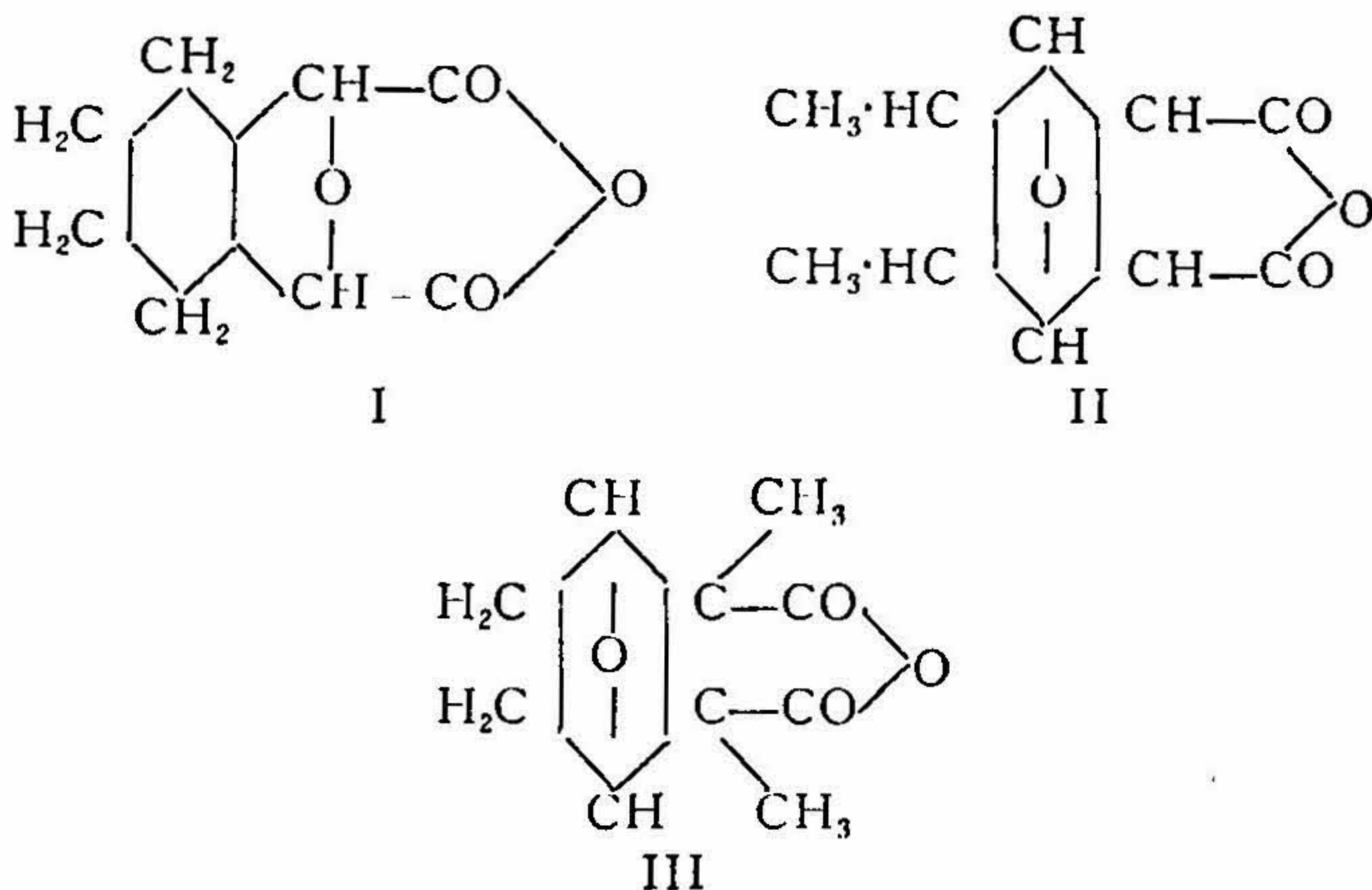
CONSTITUTION OF CANTHARIDIN, $C_{10}H_{12}O_4$.

The establishment of a constitution for cantharidin required more than thirty years (from 1892 to 1923) of laborious work, by J. Gadamer (*Arch. Pharm.*, 1914, **252**, 609 and 636; 1917, **255**, 290 and 315; 1920, **258**, 171), Walter Rudolph (*Arch. Pharm.*, 1916, **254**, 423) and Samuel Coffey (*Rec. trav. chim.*, 1923, **42**, 387 and 1026). J. Gadamer in his first communication (*loc. cit.*) has very ably reviewed the numerous results of previous workers and hence it is only necessary to give here an account of the work of Gadamer and his successors. Danckworth (*Arch. Pharm.*, 1914, **252**, 632), one of Gadamer's collaborators has shown that cantharidin dissolved in acetone neutralised more caustic potash than is required for one carboxyl group and prepared a dibasic brucine salt which could not be resolved into active forms. Thus he concluded that cantharidin is the anhydride of a dibasic acid (cantharidic acid), and definitely fixed the state of three among the four oxygen atoms in cantharidin. Gadamer in his third memoir (*Arch. Pharm.*, 1914, **252**, 636) described the action of hydrobromic acid on cantharidin, obtaining hydrobromocantharic acid and cantharidin dibromide, $C_{10}H_{12}Br_2O_3$, analogous to the diiodide of Piccard (*Ber.*, 1886, **19**, 1404). From these experiments he considered the fourth atom of oxygen to be present in an ether group, the action of halogen acids being



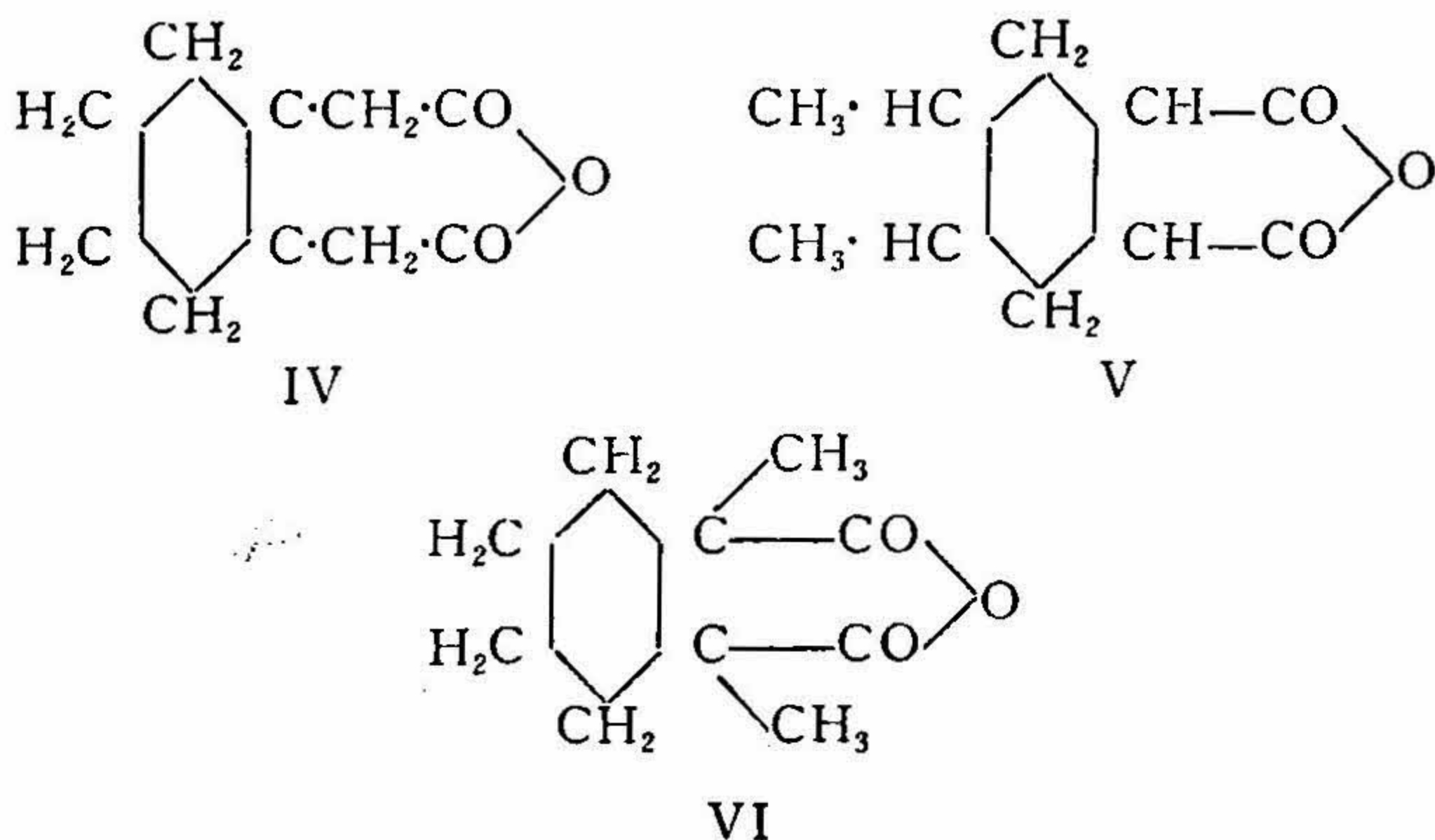
The optical inactivity of cantharidin, and the fact that active hydrobromocantharic and cantharic acids always give inactive cantharidin, led to the belief that cantharidin does not contain an asymmetric carbon atom. On treatment with hydriodic acid cantharidin yields cantharic acid, $C_{10}H_{12}O_4$, and a diiodide, $C_{10}H_{12}I_2O_3$. When this derivative is heated with a solution of caustic potash in a sealed tube, pure cantharene (dihydro-*o*-xylene) is produced, yielding *o*-toluic and phthalic acids on oxidation with nitric acid. When sodium cantharidate is distilled with soda-lime, cantharene, xylene and high boiling ketones distil (J. Piccard, *Ber.*, 1879, **12**, 577).

Based on these facts, Gadamer (*Arch. Pharm.*, 1914, 252, 624) suggested the following three formulæ for cantharidin:



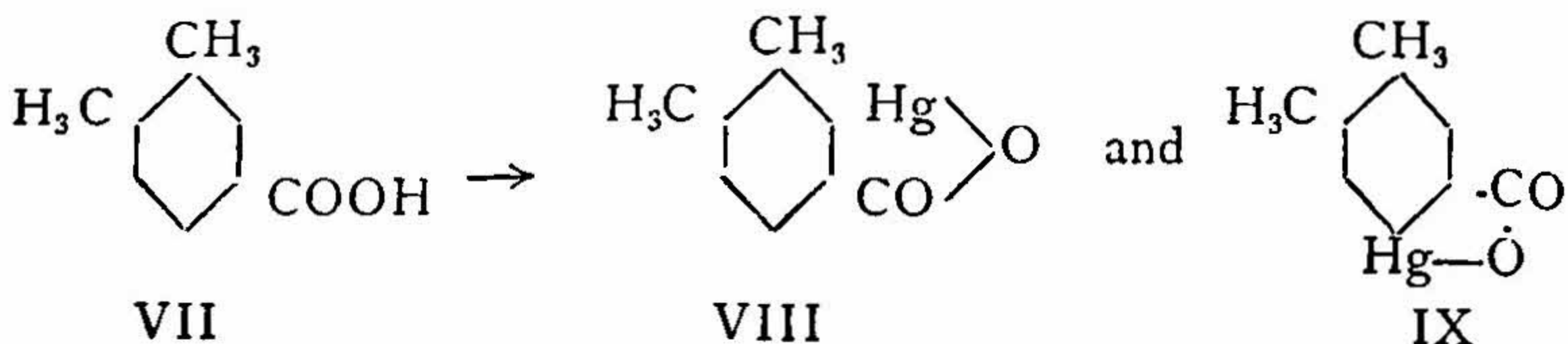
without pronouncing definitely in favour of any one, each having been found applicable to some of the reactions. At this juncture Samuel Coffey (*Rcc. trav. chim.*, 1923, 42, 387 and 1026) reviewed the past literature and made the first attack on the synthetic side.

When dibromocantharidin is reduced, deoxycantharidin arises (Rudolph, *Arch. Pharm.*, 1916, 254, 423), and according to Gadamer's formulæ might have a constitution represented by one of the formulæ,



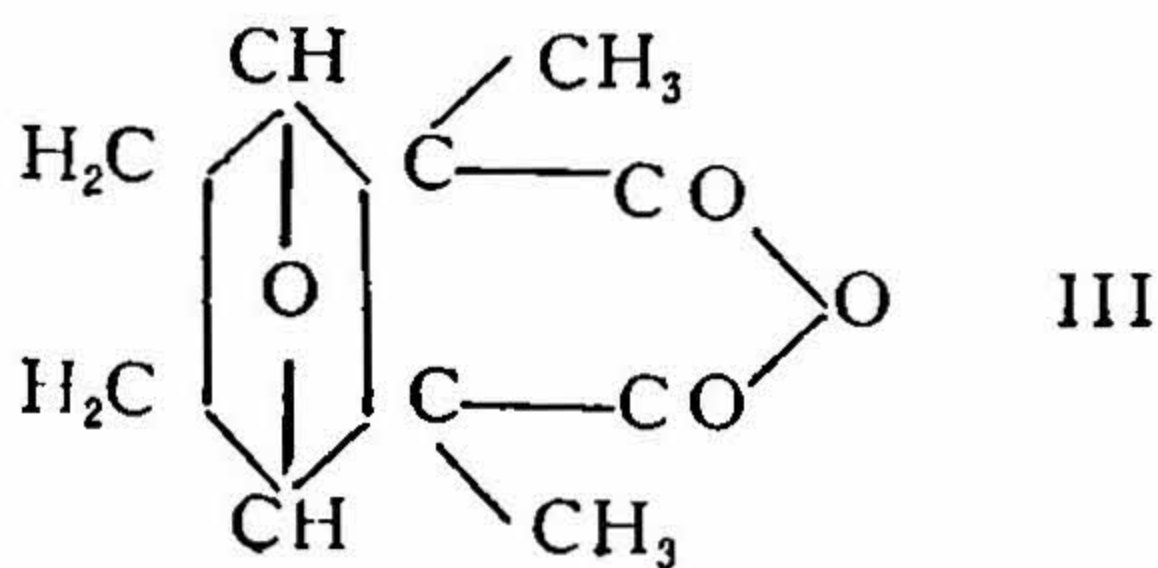
These three formulæ correspond to the names (IV) *cis-cyclohexane-1:2-diacetic anhydride*, (V) *cis-4:5-dimethylcyclohexane-1:2-dicarboxylic anhydride* and (VI) *cis-1:2-dimethylcyclohexane-1:2-dicarboxylic anhydride*.

Coffey prepared *cis-cyclohexane-1:2-diacetic acid* and its anhydride by catalytic reduction of *o*-phenylenediacetic acid, and the products being different from deoxycantharidic acid and deoxycantharidin obtained from cantharidin, formula (I) was rejected. *cis*-Hexahydro-4:5-dimethylphthalic anhydride was prepared from *p*-xylic acid, itself obtained by oxidising ψ -cumene with nitric acid (Fittig and Laubinger, *Annalen*, 1869, 151, 275; Perkin and Bentley, *J. C. S.*, 1897, 71, 166). On mercuration of *p*-xylic acid (VII) by Presci's method, Coffey produced two *o*-hydroxymercuri-*p*-xylic anhydrides (VIII) and (IX),



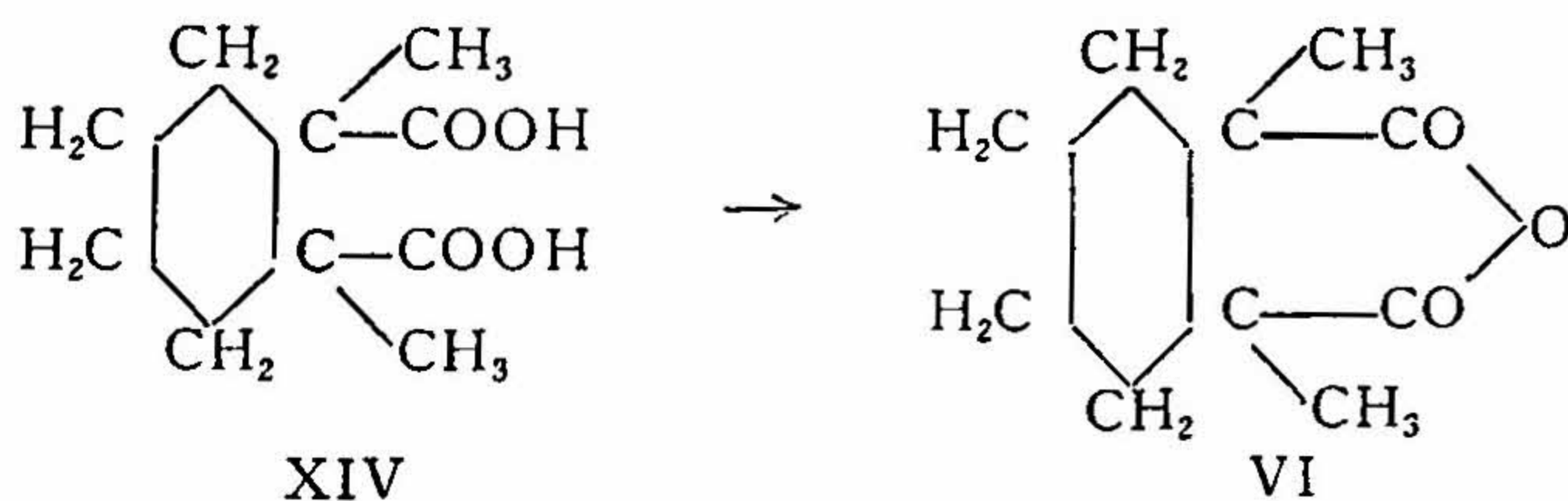
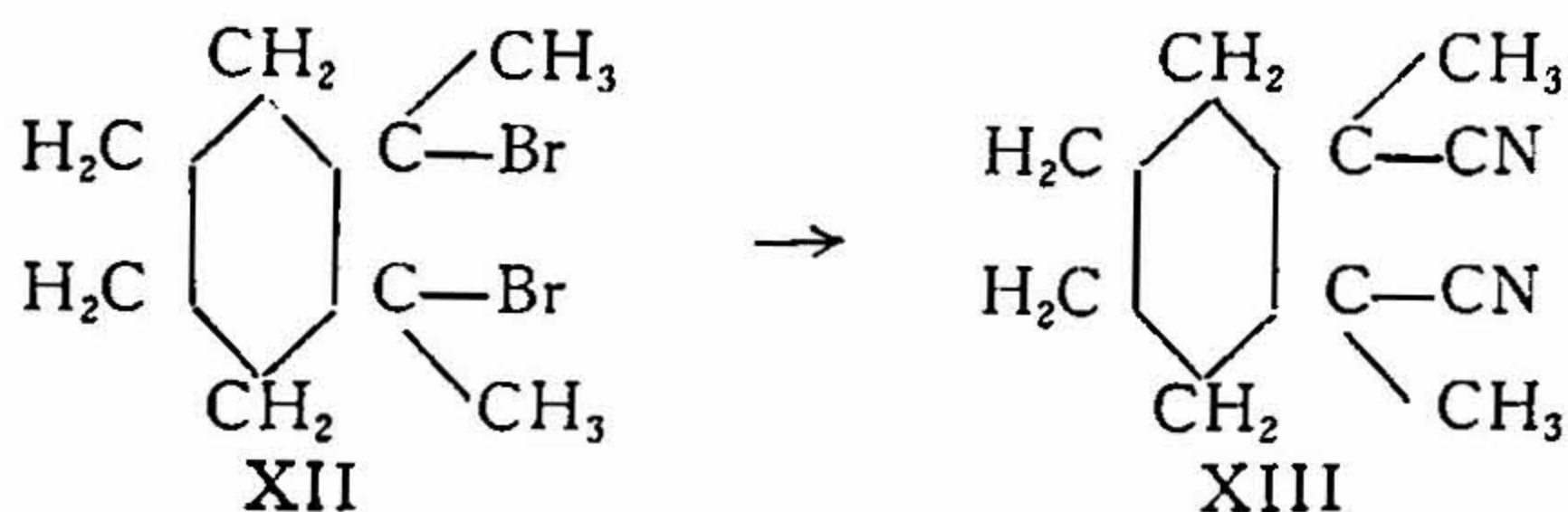
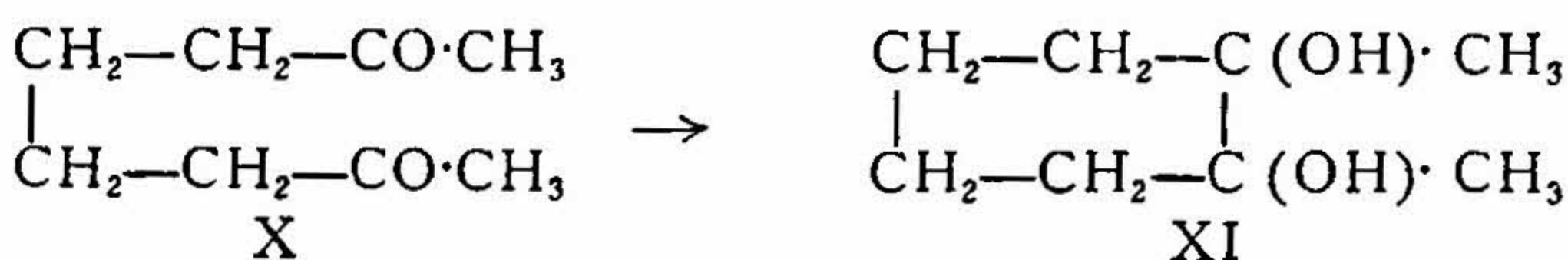
which could not be separated: hence the mixture was sulphonated, the alkali salts of the sulphonated products being easily separated by fractional crystallisation. The potassium salt of the corresponding sulpho-*p*-xylic acid on fusion with sodium formate, yielded dimethylphthalic acid which in turn was reduced by hydrogen in presence of platinum. Along with *cis*-hexahydro-4:5-dimethylphthalic acid, a small quantity of the corresponding phthalide was produced. This acid and its anhydride were not identical with the deoxycantharidic acid and deoxycantharidin, respectively; hence the second of Gadamer's three formulæ (II) was also rejected.

So by a process of elimination the third formula, to which Gadamer himself was more inclined, was confirmed, the accepted constitution for cantharidin thus being



ATTEMPTS TO SYNTHESISE DEOXYCANTHARIDIN.

A synthesis of either deoxycantharidin or cantharidin itself will be the only definite confirmation of the above. It was proposed to start with $\omega\omega$ -diacetylbutane (X) and subject it to pinacone condensation when product (XI) is to be expected. This on bromination should give 1:2-dibromo-1:2-dimethyl-*cyclohexane* (XII), yielding with potassium cyanide the dinitrile (XIII). When this is treated with acid the dicarboxylic acid (XIV) i. e., deoxycantharidic acid is to be expected, giving deoxycantharidin (VI) by dehydration.



Owing to the extreme difficulty of preparing diacetylbutane in sufficient quantity this plan had to be abandoned. However, the dibromo-compound (XII) was obtained from *o*-methylcyclohexanone (Hans Meerwein, *Annalen*, 1918, 417, 268), although for want of sufficient experimental details much difficulty was encountered. Meerwein states that the dibromide (XII) cannot be obtained in pure condition, as it immediately darkens. Although the bromination was conducted in absence of light the product would not remain pure for a reasonable period, and further difficulties were encountered on treatment with potassium cyanide. This reaction, although tried more than a dozen times under varying conditions, has always given a tarry mass from which nothing could be separated, even in absence of any hydrolytic agent. Further experiments in these lines are in progress.

EXPERIMENTAL.

COLLECTION AND PRESERVATION OF THE BEETLES.

These beetles are found in large flights between August and November; being herbivorous they are very great pests. Their habit is very sluggish during the early morning with increasing activity as the sun rises; hence the collection was made between 6 and 11 a.m.

Mylabris pustulata possess good power of flight. A yellow-coloured, bad smelling oil is exuded from the leg-joints, and the dissolved cantharidin besides causing blisters, perhaps renders them unpalatable to enemies such as birds. Beetles used in this work are black, with three orange-red bands across the elytra, and varied from 0.5 to 1.0 inch in length with average weight of 0.5 g. They emit a very acrid, nauseating odour. When alive their appearance is bright, but the orange bands lose their brilliance and the insects become dull when dead, and having very little moisture they do not decay when kept unpreserved. Collections were made from fields cultivated for cereals or vegetables in the neighbourhood of Yeshwantapur, Bangalore City, during January, February, March and April, by hand-picking. In Bangalore these insects appear throughout the year, but during the months of July, August and September they are found in greater abundance.

The insects were killed by chloroform, dried in the sun for two or three days and preserved whole or powdered. In this process, a power-driven mill was found more efficient than the mortar and pestle, which leave the wing-sheaths uncrushed. No preservatives were used excepting a few naphthalene balls. The beetles when dried lose half their original weight.

EXTRACTION OF CANTHARIDIN.

Before extraction, a qualitative test for cantharidin was made according to the method of L. David (*Pharm. Z.*, 1927, 72, 56). Three beetles were crushed in a porcelain mortar with 25 c.c. of chloroform and 5 to 10 c.c. of aqueous ammonia; after evaporation of the filtered liquid, 6 to 8 drops of fuming nitric acid were added, the residue dried and warmed with a few drops of concentrated hydrochloric acid containing vanillin (1 g. in 100 c.c.) when an orange-red coloration immediately developed indicating cantharidin.

Several methods of extraction involving different organic solvents are described in the literature. Cantharidin is markedly soluble in hot alcohol, chloroform, ethyl acetate and boiling benzene; it occurs as a salt (potassium cantharidate) as well as in the free state, so the first

step to complete extraction is to decompose the salt, either by alkali or acid. The general methods of extraction can be grouped in two categories. By the first, total cantharidin (i.e., after decomposing the salt) along with the fat in which cantharidin is very soluble is extracted with an organic solvent, either chloroform or ethyl acetate, the solvent distilled, and the fat removed either by carbon disulphide or light petroleum. In the second method, due to Puran Singh (Imp. Forest Research Inst. Reprint, 1907, 16; *J.C.S., A.*, ii, 1907, 994), an inorganic extracting medium is employed. The salt is first decomposed by alkali and the cantharidin extracted by means of a dilute solution of alum which when concentrated throws out cantharidin along with the basic salt of aluminium; this is removed by hydrochloric acid and the cantharidin crystallised from alcohol.

The method of E. Dieterich (*J. Pharm.*, 1893 (5), 27, 375; *J.C.S., A.*, i, 1893, 600) was followed with some modifications in the working details.

Experiment I (*for free cantharidin only*).—37 Grams of sun-dried and powdered cantharides were extracted in a glass soxhlet with chloroform, which was distilled after running for 24 hours; and the residue treated with cold carbon bisulphide to remove all fatty material. The crude cantharidin was crystallised from absolute alcohol and melted at 218° , not depressed by admixture with a genuine sample from Merck. The yield was 0.5 g., amounting to 1.35 per cent. of the dried beetles.

Experiment II (*for total cantharidin*).—100 Grams of dry powdered cantharides were treated with 150 g. of ethyl acetate and 2 g. of concentrated sulphuric acid; after two days 4 g. of barium carbonate was added and the mixture extracted in a glass soxhlet for 48 hours with ethyl acetate. The residue after distilling the solvent was macerated several times with cold light petroleum (b. p. $40-60^{\circ}$), the crude cantharidin being treated with a small quantity of hot alcohol and allowed to cool. By this process the small quantity of a resinous product giving a green fluorescence in alcoholic solution was removed. 46 Grams of pure cantharidin were obtained from 2 kilos of dried beetles, the yield being 2.3 per cent. From the two experiments described above, it can be seen that 0.95 per cent. of the substance exists in the form of potassium salt. Cantharidin being slowly volatile, insects kept for long periods gave smaller yields. A sample of Spanish Fly (from Europe) supplied by the Bengal Chemical and Pharmaceutical Works, Calcutta, gave practically no yield, because they might have been very old. Moreover Spanish Fly contains much less cantharidin than *Mylabris pustulata*.

The volatility of this material suggests extracting cantharidin before drying the beetles in the sun, to preclude loss. Hence the crushed insects after being subjected to sulphuric acid and barium carbonate treatment successively were extracted with chloroform. The whole process was very unpleasant and the yield was very poor, evidently due to the extraction not being efficient.

The dried insects yielded 12 to 12.5 per cent. of fat the examination of which is described in the next paper by Iyer and Ramaswami Ayyar. There it has been shown that the fatty oil still retains one more per cent. of cantharidin which could not be isolated by the usual methods, the total cantharidin content thus reaching 3.3 per cent. Although it is obtained as a by-product the fat is of very great value as it could be used as such for external applications of cantharidin.

SOME REACTIONS OF CANTHARIDIN.

In attempts to elucidate the constitution of cantharidin, various derivatives and reactions have already been studied. In some cases, especially with phenylhydrazine, several compounds were obtained. As the constitution was not known at that time, no definite explanation has been offered.

The action of 4-phenylthiosemicarbazide, urea, guanidine hydrochloride and hydrazine hydrate on cantharidin has now been tried, but no reaction could be observed in the first three cases, either in alcoholic solution or by heating a mixture of the two substances at a high temperature.

Cantharidin Hydrazide.—To 5 g. of cantharidin mixed with a few c.c. of absolute alcohol was added under ice cooling an alcoholic solution containing 3.75 g. (twice the theoretical quantity) of hydrazine hydrate. There did not appear to be any change until room temperature was reached when the reaction started, and on keeping overnight the whole quantity of the solid went in solution. After boiling this for an hour on a water bath, alcohol was distilled and the solid crystallised from alcohol, melting at 118°. The hydrazide is very soluble in water, ether, acetone, and is also soluble in alcohol, hot petroleum (b.p. 50-60°), benzene, dilute acetic and hydrochloric acids. Yield, 5.5 g., i.e., 80 per cent. of theory (Found: C, 56.83; H, 5.72; N, 13.72. $C_{10}H_{14}O_3N_2$ requires C, 57.14; H, 6.67; N, 13.33 per cent).

Benzylidene derivative.—One gram of the hydrazide dissolved in water was well shaken with 0.7 g. of benzaldehyde and then heated on a water bath for half an hour. On slow cooling crystals separated,

and after recrystallisation from 98 per cent. alcohol, melted at 158° (Found: N, 9.78. $C_{17}H_{18}O_3N_2$ requires N, 9.39 per cent.).

The preparation of $\omega\omega'$ -diacetylbutane was tried according to Marshall and Perkin (*J.C.S.*, 1890, 57, 24) their method being the only one known; but although the experiment was repeated over a dozen times no workable quantity could be obtained. Consequently experiments were conducted on the electrolysis of potassium laevulinate according to Hans Hofer (*Ber.*, 1900, 33, 650), who has not given any experimental detail as to the electrodes; several experiments with nickel and carbon electrodes were tried, but were unsuccessful.

Preparation of 1:2-Dimethylcyclohexene.

Following Meerwein (*Annalen*, 1914, 405, 145), the scanty experimental details allowed only a poor yield to be obtained in the first few experiments; improvement resulted from the following modifications. 100 Grams of freshly fused and well powdered zinc chloride were heated, in a round bottomed flask fitted with a long air condenser protected by a calcium chloride tube, to 120° on an oil bath. On cooling to 90° , 50 c.c. of 1:2-dimethylcyclohexanol were added and the temperature raised to $170-180^{\circ}$ for 10 to 15 minutes. Further 15 g. of zinc chloride was added and the product rapidly distilled, dried over calcium chloride and redistilled, when 32 g. of the pure hydrocarbon (b.p. $132-134^{\circ}$) was obtained. Heating the dimethylcyclohexanol for a longer time invariably gave an inferior yield.

CONCLUSION.

The maximum yield of cantharidin hitherto recorded is 1.9 per cent. from Chinese beetles, but the foregoing experiments having shown a yield of 2.3 per cent. it is evidently quite feasible to produce it in India. Moreover, the cost of laboratory-production, representing labour of collection and value of materials without deduction for recovery of ethyl acetate, amounts roughly to 6 annas per gram, which compares very favourably with the cost of imported cantharidin at Rs. 2-3-0 per gram.

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