

## SCIENTIFIC SECTION

### DERIVATIVES OF PARA-METHOXYCINNAMIC ACID.\*

BY P. A. FOOTE.

In order to obtain a sufficient amount of *n*-pentadecane, discovered by van Romburgh (1), it was necessary to secure a somewhat large amount of the volatile oil from the rhizome of *Kaempferia Galanga L.*

This was specially distilled at the Botanical Garden at Buitenzorg, Java. To the Director and his associates our sincere thanks for their kind coöperation are herewith publicly expressed. Inasmuch as the oil consists largely of *p*-methoxycinnamic acid ethyl ester, which had to be removed before the hydrocarbon could be isolated, a considerable quantity of the free acid became available. It is a handsomely crystalline compound. The temptation to do something with it was too great to be neglected.

*Preparation of Salts of p-Methoxycinnamic Acid.*—Perkin (2) has prepared the salts of the following metals: Na, Ca, Ba, Sr, Cu, Fe and Ag. van Romburgh (3) has prepared the salts of K and Ag.

The Na and K salts may be prepared by saponification of the ethyl ester. These being water soluble the salts of the heavy metals and alkaline earths may be readily prepared from them by double decomposition, for the latter are insoluble in water. By this method all the above salts have been prepared except that of Sr. In like manner salts of Hg<sup>''</sup>, Zn and Au<sup>'''</sup> were prepared for the first time. The Hg<sup>''</sup> and Zn salts are white and amorphous. The Au<sup>'''</sup> salt is at first yellow but soon turns a dark brown.

The ammonium salt was prepared by passing dry NH<sub>3</sub> into a hot toluene solution of the acid. Within 20 minutes the salt begins to precipitate in the form of beautiful white flakes. It is soluble in water, alcohol and acetone.

*Preparation of New Esters.*—The use of cinnamic acid and its derivatives in the fields of medicine, perfumery and preservation suggested the preparation of new esters of the *p*-methoxy acid.

*Phenyl Ester.*—Five-Gm. *p*-methoxycinnamic acid plus 5-Gm. phenol plus 2.4-cc. phosphorus trichloride were refluxed for 2 hours in toluene (dried over sodium) on a water-bath. The toluene was distilled off under diminished pressure and the residue washed with 5 p. c. aqueous KOH to remove acids and phenol. The product was then washed with water until neutral to litmus, brought on a suction filter and dried in a desiccator over night. Crystallized from acetone it was obtained as white crystals m. p. 76-77° C. Yield, 4.5 Gm. The ester is insoluble in water but soluble in the common organic solvents.

When two 0.2-Gm. samples were saponified with *N*/2 alcoholic KOH an average ester value of 217.5 was found. The theoretical value is 220.9. The mother liquids were combined and after evaporating off the alcohol the *p*-methoxycinnamic acid was precipitated by HCl, filtered, dried and identified by its m. p. 171° C. The phenol was identified in the filtrate as tribromphenol.

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*β-Naphthyl Ester*.—By way of experience the cinnamate was first prepared, the m. p. of which (101–102°) checked with that found by Anschuetz (4) who first prepared it from the acid chloride.

*β-naphthol*, 4.07 Gm., plus 5 Gm. of *p*-methoxycinnamic acid plus 2.4 cc. of phosphorus trichloride were refluxed in toluene (dried over sodium) on a water-bath, then on an oil-bath at 110–120° C. for a total of two hours. The toluene was distilled off under diminished pressure on a water-bath and the residue washed with 5 p. c. aqueous KOH to remove free phenol and acid, then with water, dried on a suction filter and then in a desiccator. Yield, 7.1 Gm. The ester was crystallized from a mixture of equal parts of acetone and alcohol. Flesh-colored flaky crystals m. p. 130–131° C., resulted. These were recrystallized from benzene in colorless crystals with no change in the melting point.

The ester value determined with *N/2* alcoholic KOH was found to be 204.1. The theoretical value is 184.5. The alcohol from two combined mother liquids was removed by evaporation. The *β-naphthol* was shaken out with ether and identified by the KOH and CHCl<sub>3</sub> method (U. S. P. X). The acid was precipitated by HCl, filtered, dried and identified by its m. p. 171° C.

*Thymyl Ester*.—In this case also the cinnamate was first prepared for the sake of experience. It melted at 69–70°. Anschuetz (4) records the same melting point but prepared his ester from the acid chloride.

Five Gm. of thymol plus 5 Gm. of *p*-methoxycinnamic acid plus 2.4 cc. of phosphorus trichloride were refluxed in toluene (dried over sodium) for 2 hours on a water-bath. The toluene was distilled off under diminished pressure on a water-bath and the residue washed with 5 p. c. aqueous KOH, dried on a suction filter and then in a desiccator. The product was crystallized from 95 p. c. alcohol giving beautiful long white needles m. p. 58–59° C. The ester is insoluble in water but soluble in the ordinary organic solvents.

When saponified with *N/2* alcoholic KOH an average of two samples gave an ester value of 179.2, the theoretical value being 180.9. The two mother liquids of the samples were combined and acidified to precipitate the *p*-methoxycinnamic acid which was identified by its m. p. 171° C. A steam distillate of the mother liquid gave the Flueckiger test for thymol.

*Carvacryl Ester*.—Fifteen cc. of carvacrol plus 10 Gm. *p*-methoxycinnamic acid plus 7.65 Gm. (4.75 cc.) phosphorus trichloride were refluxed in toluene previously dried over sodium for two hours on an oil-bath at 130° C. The toluene was distilled off under diminished pressure on a water-bath and the residue washed with 5 p. c. aqueous KOH, dried on a suction filter and then in a desiccator. The product was crystallized from ether in a desiccator over H<sub>2</sub>SO<sub>4</sub>, the ester appearing in large colorless crystals, m. p. 78–79° C. Yield, 7.5 Gm. It is insoluble in water but soluble in the ordinary organic solvents.

When saponified with *N/2* alcoholic KOH two 0.2-Gm. samples gave an average ester value of 179.3, the theoretical value being 180.97. The products of hydrolysis were identified in a similar manner to those of the thymyl ester.

*Eugenyl Ester*.—For the sake of experience, the cinnamic ester was first prepared. This is covered by D.R.P. 68,111 but information as to its preparation is wanting. Inasmuch as the patent does not record the m. p. of the cinnamic ester, it was determined and found to be 90–91°.

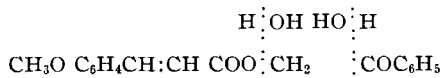
Seven- and five-tenths Gm. of eugenol plus 5-Gm. *p*-methoxycinnamic acid plus 2.4-cc. phosphorus trichloride were refluxed in dry toluene solution for 2 hours on an oil-bath. The toluene was distilled off under diminished pressure on a water-bath and the residue washed with 5 p. c. aqueous KOH to remove acids and eugenol. The product was then washed with water until neutral to litmus, brought on a suction filter and finally dried in a desiccator. When crystallized from a 50-p. c. alcohol-50-p. c. acetone solution the ester was obtained in large white crystals m. p. 112-113° C. Yield, 5.1 Gm. It is insoluble in water but soluble in the common organic solvents.

Two samples gave an average ester value of 171.0, the theoretical value being 173.1. To identify the products of hydrolysis the mother liquids were combined, the alcohol evaporated, the eugenol extracted with ether and identified by the FeCl<sub>3</sub> color test. The *p*-methoxycinnamic acid was precipitated by HCl, filtered, dried and identified by its melting point 171° C.

*Phenacyl Ester*.—This was prepared by refluxing in an aqueous alcoholic solution 4.97 Gm. of  $\omega$ -bromacetophenone (C<sub>6</sub>H<sub>5</sub>CO CH<sub>2</sub>Br) and 5 Gm. of the sodium salt of the acid. Enough water was used to dissolve the sodium salt and enough 95-p. c. alcohol to dissolve the  $\omega$ -bromacetophenone. After 1½ hours the mixture was allowed to cool. A white precipitate formed, the amount of which was increased by distilling off the alcohol. This was filtered, washed with water and crystallized from acetone. Yield 3.5 Gm., m. p. 136° C. It is insoluble in water but soluble in most organic solvents.

When saponified with *N*/2 alcoholic KOH, two 0.5-Gm. samples gave ester values of 448 and 435. The theoretical value for one molecule of KOH per molecule of ester is 189.5. When repeated using two 0.2-Gm. samples with the same quantity (25 cc.) of *N*/2 alcoholic KOH the ester values found were 650 and 620, respectively. The discrepancies may be due to the formerly observed fact that under the influence of KOH, phenacyl alcohol is converted to benzaldehyde (5). The mother liquid has a faint benzaldehyde odor and gives a positive fuchsine test. The values found for the second two samples may be due to a greater proportion of KOH used per Gm. of ester, producing a higher p. c. of benzaldehyde or other changes in the same length of time (1 hr.).

Inasmuch as the odor of benzaldehyde suggests the "Abbau" of the regenerated phenacyl ester as indicated in the following formula:



it would seem possible that methylene glycol or formic aldehyde may be formed at the same time. It is well known that in the presence of strong KOH benzaldehyde undergoes autooxidation as well as autoreduction even at room temperature. It might well be assumed that heat applied to such a reaction mixture, even if the KOH be weaker, would produce a similar result. This would account for an increase of 50 p. c. of the S. V. If it be assumed that formaldehyde under like conditions, *i. e.*, in the nascent state as it were, may undergo a like reaction of autooxidation and reduction to formic acid and methyl alcohol, respectively, another increase of 50 p. c. in the S. V. could be accounted for. The two CO groups

in the original molecule would seem to render such hydrolysis products rather probable.

*Monoxime of Phenacyl Ester.*—Twenty Gm. of the phenacyl ester plus 16 Gm. of hydroxylamine hydrochloride plus 16 Gm. of  $\text{Na}_2\text{CO}_3 \cdot \text{H}_2\text{O}$  were refluxed for one hour in a 95-p. c. alcohol solution. A large portion of the alcohol was distilled off and replaced by water giving a white precipitate, which when dried on a suction filter and then in a desiccator over night gave a m. p. of 115–116°. Recrystallized from 95-p. c. alcohol it gave a m. p. of 116–117° C. The yield was 13.2 Gm. A Kjeldahl nitrogen determination on two 0.2-Gm. samples gave 4.46 p. c. and 4.80 p. c. The theoretical amount is 4.5 p. c. Therefore, the compound contains one nitrogen atom.

When saponified with *N/2* alcoholic KOH two 0.2-Gm. samples gave ester values of 262.2 and 268.5. The theoretical value for 1 molecule of KOH per 1 molecule of the oxime is 180.4. As in the case of the phenacyl ester the mother liquid had an odor of benzaldehyde and gave a positive fuchsine test. An attempt to acetylate the oxime resulted in its hydrolysis to the phenacyl ester.

As already stated, the cinnamic esters were prepared by way of experience. Inasmuch as one of these was thus prepared for the first time it is, herewith, described.

*Carvacryl Cinnamate.*—Ten Gm. of cinnamic acid chloride were slowly added to 10 cc. of carvacrol and the mixture heated on a water-bath for one hour. The product was washed with 5-p. c. aqueous KOH to remove free acid and carvacrol. The amorphous slightly yellow product was washed with water, dried on a suction filter and crystallized from 95-p. c. alcohol. Yield, 5.0 Gm. The ester crystallized in long, white needle-like crystals, m. p. 65–66° C. It is insoluble in water but soluble in the ordinary organic solvents.

When saponified with *N/2* alcoholic KOH two 0.2-Gm. samples gave an average ester value of 201.8, the theoretical value being 200.0. The two mother liquids of the samples were combined and acidified to precipitate the cinnamic acid which was identified by its m. p. 131° C. A steam distillate of the mother liquid gave the Flueckiger test for carvacrol.

*Antiseptic Properties of These Esters* (6).—Landerer's discovery (7) that cinnamic acid was supposed to be beneficial in tuberculosis aroused an interest in its derivatives, for Ehrlich and his co-workers had about the same time initiated the science of chemotherapy. Hence many cinnamic acid derivatives have been prepared but none of them have an outstanding value in medicine to-day. Prompted by this work the following antiseptic tests were made on five phenols, their cinnamates and *p*-methoxycinnamates. Each phenol test being made for comparison with that of its esters. The phenols employed were phenol,  $\beta$ -naphthol, thymol, carvacrol and eugenol.

The compounds were tested against *Bacillus typhosus* and *Bacillus staphylococcus* representing Gr. positive and Gr. negative types of bacteria. Three drops of a 24-hour broth culture were spread over agar in Petri dishes, a few crystals or drops of the compound were placed thereon. After 48 hours incubation at 37° C. the bacterial growth was noted. A toxic substance should inhibit the growth as the phenols did. Duplicate tests were made but the esters showed no such property.

That the acid itself has little or no toxicity was shown by intravenous and subcutaneous injection into white rats. A 2-p. c. aqueous solution of the sodium salt was used in doses of 250, 310 and 420 mg. per kilo body weight. No outward symptoms of toxicity could be detected.

## REFERENCES.

- (1) *Könl. Akad. Wet. Amst.*, 4, 618.
- (2) *J. Chem. Soc.* [3], 1, 388.
- (3) *I. c.* 3, 38.
- (4) *Ber.*, 18, 1946.
- (5) Reed, who developed this reagent for identification of acids does not give ester values or a clue to such variations.
- (6) The bacteriological tests were made under the direction of Prof. P. F. Clark in the School of Medicine.
- (7) *Behandlung der Tuberkulose mit Zimtsaeure*, Leipzig (1898).

## BIO-ASSAY OF PREPARATIONS OF OVARIAN FOLLICULAR HORMONE.\*

BY E. P. BUGBEE AND A. E. SIMOND.

The Ovarian Follicular Hormone is the female sex hormone which is obtained from the fluid contained in ovarian follicles, from whole ovaries, placentas and other female tissues. In France and Germany it is known as Folliculin and in England as Estrin because it produces oestrus or heat in animals.

Preparations containing Ovarian Follicular Hormone are assayed for biological activity by injecting the solutions into white rats from which the ovaries have been completely removed. If adequate doses are injected the rats will come into oestrus and show the same evidences of heat that normal rats show when in that condition.

The most accurate way for the laboratory worker to tell when the rats are in heat is by examination of vaginal smears. For this method we are indebted to Stockard and Papanicolaou (1), who in 1917 discovered that in guinea-pigs the vaginal mucous membrane goes through the same growth changes as the uterine mucous membrane in each oestrous cycle. Long and Evans (2) soon confirmed this in their studies with rats and Allen (3) confirmed it in his studies with mice.

Small cotton swabs are inserted gently into the vaginas and then glass slides are smeared with the swabs. After drying, the slides are stained with haematoxylin and eosin to differentiate the cells. They are examined under the microscope to determine the types of cells present. Certain types characterize the 5 stages of the oestrus cycles in rats. These have been classified by Long and Evans (2) as follows:

Stage I—Pro-oestrus. Before heat begins. No sexual excitement. Vaginal smears show small epithelial cells with nuclei. No leucocytes.

Stage II—Oestrus. The heat period. Copulation accepted. Vaginal smears show large squamous cells without nuclei. No leucocytes.

Stage III—Late Oestrus. The heat period is over. No sexual excitement. Vaginal smears are thick and cheesy, containing clumps of large squamous cells without nuclei. Late in this stage there may be some large epithelial cells with nuclei. There are no leucocytes.

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\* Scientific Section A. PH. A., Portland meeting, 1928.