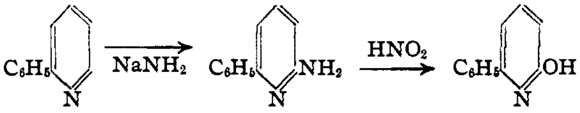
lized best from methanol in flat rods with square ends, m. p. 195°; although it appeared to crystallize well from petroleum ether the melting point was lower by 10°. A hydrochloride of m. p. 101–103° was prepared as directed in the literature, which gave the melting point 104°. All the properties agree with those described by Leben.[13] In order to establish the identity with certainty, a specimen was prepared as directed in the literature,[14,15] the melting point was not depressed on admixture.

Owing to the roundabout method of synthesis, several attempts were made to obtain it by a simpler procedure, but without success. It was relatively easy to prepare 2-aminopyridine and 2-phenylpyridine but impossible to introduce both groups into the same molecule. The action of sodium amide on 2-phenylpyridine led to a high melting, bimolecular product.



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## Summary

$\gamma$-Benzoylbutyronitrile has been prepared by the dehydration of the corresponding amide with acetic anhydride. Other dehydrating agents gave a cyclic, isomeric derivative of tetrahydropyridine. The characteristic properties of each have been determined.

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[13] Leben, *Ber.*, **29**, 1673 (1896).

[14] Kalff, *Rec. trav. chim.*, **46**, 594 (1927).

[15] In this work we were assisted by Mr. J. C. W. Evans. Some of the preliminary steps were first carried out by Dr. A. C. Bell. Their aid is gratefully acknowledged.

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[CONTRIBUTION FROM THE ORGANIC RESEARCH LABORATORY, CHEMISTRY DEPARTMENT, UNIVERSITY OF THE PHILIPPINES]

# On the Naturally Occurring Linolic Acid in Cottonseed and Soya Bean Oils and the Regenerated Linolic Acid from Alpha Linolic Acid Tetrabromide of These Oils

BY D. M. BIROSEL

In a previous paper,[1] it was reported that only two isomeric linolic acid tetrabromides were isolated from the products of direct bromination of the free fatty acids of lumbang or candle nut (*Aleurites moluccana*) oil and of the regenerated linolic acid; that only two tetrahydroxystearic acids were isolated from the products of oxidation with alkaline permanganate solution of the free fatty acids of lumbang oil and of the regenerated linolic acid; and that the $\beta$- and $\delta$-linolic acid tetrabromides which have been reported by other workers as obtainable from the bromination product of the free fatty acids of lumbang oil are ethyl ester mixed with varying amount of free solid acid tetrabromide.

Considerable work[2] has been done on linolic acid but the literature presents conflicting observations and conclusions. The present work is primarily intended to supplement the observations previously recorded by the writer. It is desired to report in this paper the observations made on linolic acid naturally present in soya bean and in cottonseed oils and on the regenerated linolic acid from $\alpha$-linolic acid tetrabromide prepared by direct bromination of the free fatty acids of these oils.

From the direct bromination of the dried free fatty acids of soya bean and cottonseed oils, using ether as reaction medium and low boiling point petroleum ether[3] as vehicle of crystallization, $\alpha$-linolic acid tetrabromide (m. p. 114°) was the only

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[1] Birosel, *Natural and Applied Science Bulletin of the University of the Philippines*, **2**, 103 (1932).

[2] Meyer and Beer [*Monatsh.*, **33**, 325 (1912)] obtained $\beta$-sativic acid of m. p. 174° and $\alpha$-sativic acid of m. p. 163° from the oxidation with cold alkaline permanganate solution of the free fatty acids of "Jimson weed" *Datura Stramonium.* Nicolet and Cox [THIS JOURNAL, **44**, 144 (1922)] doubted the existence of these sativic acids on the ground that the fatty acids of Jimson weed seeds yield $\alpha$-linolic acid tetrabromide, m. p. 117°, whereas the highest melting point recorded in the literature is 114°. A. P. West and co-workers [*Philippine J. Sci.*, **32**, 41 (1927); *cf. C. A.*, **21**, 1720 (1927); *ibid.*, **32**, 297 (1927); *cf. C. A.*, **21**, 2250 (1927); *ibid.*, **42**, 251 (1930)] reported having isolated three solid linolic acid tetrabromides from the free fatty acids of lumbang oil. They claimed that their $\beta$-linolic acid tetrabromide corresponds to the compound which was reported by Takahashi [*J. Tokyo Chem. Sci.*, **4**, 233 (1919); *C. A.*, **13**, 1583 (1919)] but regarded their $\alpha$-linolic acid tetrabromide of lumbang oil as different from a similar compound from soya bean oil on the ground that their regenerated linolic acid did not yield the $\beta$-linolic acid tetrabromide of Takahashi. Birosel[1] has shown that the $\beta$- and $\delta$-linolic acid tetrabromides of West are ethyl ester of the acid mixed with varying amount of free $\alpha$-linolic acid tetrabromide.

[3] Birosel[1] reported that with ethyl alcohol as vehicle of crystallization for the crude solid acid tetrabromide, the $\beta$- and $\delta$-acids of West[2] were obtained whereas if low boiling petroleum ether was used none was obtained. West's[2] compounds were also obtained when pure $\alpha$-linolic acid tetrabromide, m. p. 114°, was refluxed with ethyl alcohol until oil separated upon cooling the solution. The supposedly $\beta$- and $\delta$-acids did not give neutralization value but their alcoholic solutions to which a few drops of phenolphthalein solution were added turned red upon addition of the first drop of standard alkali.

solid tetrabromide[4] isolated from the bromination products.

When the linolic acid regenerated from the α-linolic acid tetrabromide prepared from the free fatty acid of soya bean oil by direct bromination, was subjected to the action of bromine in the proper way, and using petroleum ether as crystallization medium, α-linolic acid tetrabromide,[5] m. p. 114°, was the only solid product obtained. No solid acid tetrabromide of lower melting point was isolated.

On oxidizing the free fatty acid of soya bean and cottonseed oils with cold alkaline permanganate solution, only two tetrahydroxystearic acids[6] were isolated. The corresponding acids prepared from the two oils have identical melting points.

Oxidation with cold alkaline permanganate solution of the regenerated linolic acid from the α-linolic acid tetrabromide (m. p. 114°) prepared from the free fatty acids of cottonseed and soya bean oils by direct bromination gave α-sativic acid (m. p. 164°) and β-sativic acid (m. p. 174°).

It is thus seen that the observations previously made by the writer on the naturally occurring linolic acid in lumbang oil and on the regenerated linolic acid from α-linolic acid tetrabromide prepared from the said oil by direct bromination find support in the experimental observations recorded in this paper.

## Experimental Part

**Soya Bean Oil.**—This oil was prepared from soya bean seed by extraction with ether in the usual way. A liter of oil was prepared.

**Preparation of Free Fatty Acids of Soya Bean Oil.**—Five hundred grams of soya bean oil was treated in the usual way with a little more than the equivalent amounts of potassium hydroxide in aldehyde-free alcohol solution. When a homogeneous product after digestion on the steam-bath was obtained, the alcohol was distilled off and the soap solution was treated with excess dilute sulfuric acid. The mixture was digested to complete hydrolysis. The oil which floated was separated and taken with ether and washed several times with water until the washing was neutral. The ethereal solution was dried with anhydrous sodium sulfate, after which it was filtered and the solvent distilled. The residue which was free from solvent was further dried in a desiccator.

**Preparation of α-Linolic Acid Tetrabromide.**—Two hundred and twenty-eight grams of the dried free fatty acid of soya bean oil was dissolved in two liters of dry ether in a three-necked, round-bottomed flask of three-liter capacity. The solution was cooled down to −10°, with ice–salt mixture. Sixty-five cc. of bromine was added slowly while the solution was stirred mechanically. The temperature was not allowed to rise above 0°. Bromination took four hours to complete. Due to excess bromine, the solution was orange in color. The ether-insoluble precipitate was filtered off and washed with the same solvent. The filtrate and washing were washed together with dilute sodium thiosulfate solution until the color disappeared. The ethereal solution was dried with anhydrous sodium sulfate and filtered. The solvent was distilled off and the residue was taken up in low boiling petroleum ether from which the α-linolic acid tetrabromide separated on cooling. The compound was found to melt at 111–113°. Two recrystallizations in the same solvent increased the melting point to 114°.

After exhausting the mother liquor of its α-linolic acid tetrabromide content, it was worked out for other solid tetrabromides of lower melting points. None was isolated from the mother liquor and from the concentrated filtrate of recrystallization.

**Preparation of Linolic Acid.**—Twenty grams of the α-linolic acid tetrabromide (m. p. 114°) was refluxed with zinc in 8% alcoholic solution of hydrochloric acid for seventy-two hours continuously. The ester of the regenerated unsaturated acid was saponified with excess potassium hydroxide and the soap solution was hydrolyzed with dilute hydrochloric acid. The oil that separated out was taken up in ether and the ethereal solution was washed several times with distilled water and then dried with anhydrous sodium sulfate, after which it was filtered and washed with anhydrous ether. The filtrate and washing were distilled together, after which the residue was dried in the desiccator. In all these operations care was exercised to prevent loss of product. The dried residue weighed 9.662 g. From the figures the percentage yield and purity of the product is 98%. This percentage was checked by the result of a subsequent reduction.

**Linolic Acid Tetrabromides from the Regenerated Linolic Acid.**—9.55 grams of the regenerated linolic acid was brominated as described above. Care was exercised to avoid missing products previously reported by Takahashi.[5] Using petroleum ether as vehicle of crystallization, I obtained from the first crystallization a product which melted sharply at 114°. The amount of α-linolic acid tetrabromide was 8.8 g., which represents 45% of the regenerated linolic acid used. No solid tetrabromide of lower melting point was isolated from the mother liquor from which liquid γ-linolic acid was obtained.

**Oxidation of the Free Fatty Acids of Soya Bean Oil.**—Fifteen grams of the dried fatty acids of soya bean oil was oxidized with cold alkaline permanganate solution according to the procedure of Rollet.[7] The acid was treated with a little excess more than the equivalent amount of potassium hydroxide and the soap was dissolved in two liters of water. Twenty-two grams of potassium permanganate was dissolved and made up to one liter of solution. This

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(4) Reformatzky, *J. prakt. Chem.*, **41**, 529 (1890); Hazura, *Monatsh.*, **9**, 180 (1888), and others, obtained only one solid linolic acid tetrabromide on brominating linolic acid.

(5) Takahashi[2] obtained β-linolic acid tetrabromide, m. p. 60°, from the regenerated linolic acid of α-linolic acid tetrabromide of soya bean oil. When petroleum ether was used as vehicle of crystallization as was done in this work no β-acid tetrabromide was isolated.

(6) Meyer and Beer[2] and Birosel[1] have obtained only α-sativic acid, m. p. 163°, and β-sativic acid, m. p. 174°, by directly oxidizing the free fatty acids of the oils which they studied with cold alkaline permanganate solution.

(7) A. Rollet, *Z. physiol. Chem.*, **62**, 410 (1909).

solution was added slowly to the soap solution which was shaken after each addition. After a quarter of an hour when the last drop of permanganate solution was added, sulfur dioxide was passed into it until the precipitate in the flask became white. The precipitate was filtered off and washed until free from acid. It was dried and then extracted with ether. The ether insoluble residue weighed 11.2 g. and was found to melt at 155–160°. After washing several times with hot benzene the compound was found to melt at 160–168°. The solid was then taken up in hot water. Ten hot water extractions were made. Each one of the first eight extractions was 1 liter while each of the last two was 2.5 liters. On cooling the solutions, a white solid precipitated which was separately filtered. Ten fractions were then collected.

TABLE I

FRACTIONS AND THEIR MELTING POINTS

| Fractions | M. p., °C. | Fractions | M. p., °C. |
|---|---|---|---|
| 1 | 160–170 | 6 | 155–160 |
| 2 | 160–170 | 7 | 155–158 |
| 3 | 160–170 | 8 | 165–171 |
| 4 | 155–158 | 9 | 165–171 |
| 5 | 156–160 | 10 | 167–172 |

The various fractions were combined to form three new fractions. Fractions 1–3 were designated as fraction A; 4–7, fraction B; 8–10, fraction C.

Fraction A was washed with hot benzene and then recrystallized in hot alcohol. A solid melting at 155 to 160° was obtained. It was added to fraction B which was washed with hot benzene. The residue of B was crystallized in hot alcohol and while the solution was still warm a fraction was obtained which melted at 156 to 158°. On reducing the volume of filtrate, a second crop of solid was obtained which melted at 163°. The first crop of B was recrystallized fractionally in hot alcohol and two crops of solid were obtained; the first crop melted at 168–171° and a second crop at 163°. The higher melting portion was added to C fraction and together were washed with hot benzene, and fractionally crystallized in hot alcohol. The solid in the warm solution was filtered off and found to melt sharply at 174°. On reducing the volume of the filtrate a second crop was obtained which melted at 174°.[8]

All the residual filtrates from the various fractional crystallizations were added and reduced in volume and upon cooling a solid compound was obtained which melted at 155–158°. It was subjected to the same fractional crystallization and the same results were obtained. A small amount of residual solid remained melting at 156–158°. This was undoubtedly a mixture of the two sativic acids. When the fraction melting at 163° was recrystallized, its melting point was raised to 164°.

**Oxidation of the Regenerated Linolic Acid of Soya Bean Oil.**—4.55 grams of the regenerated linolic acid was oxidized as described above with cold alkaline permanganate solution and 1.4 g. of hot water-soluble compound was obtained. The dried compound was extracted with ether and there remained 1.25 g. of ether-insoluble residue which was found to melt at 158–162°. None dissolved in hot benzene. It was fractionally crystallized as above and the first fractional crystallization gave two fractions, one melting at 166–169° (fraction A) and the other at 159–161° (fraction B). Fraction A on fractional crystallization gave fractions melting at 165–168° and at 161–163°. The lower melting fraction was added to B and upon fractional crystallization the mixture gave $\alpha$-sativic acid, m. p. 164°. The fraction melting at 165–168° was fractionally crystallized also. The solid obtained from the filtration of the warm solution was found to melt sharply at 174°. The residual filtrates of crystallization were added together and reduced in volume. From the solid obtained two sativic acids were isolated, but a small residue melting at 156–158° remained which was undoubtedly a mixture of the two acids. Oxidation was repeated and the same results were obtained.

**Free Fatty Acids of Cottonseed Oil.**—The free fatty acids of cottonseed oil were prepared in the same way as the free fatty acids of soya bean oil. The oil is from a stock of commercial oil in the Department of Chemistry, University of the Philippines.

**Oxidation of the Free Fatty Acids of Cottonseed Oil.**—Thirty grams of the dried fatty acids from cottonseed oil was oxidized in the same manner as previously described for the fatty acids of soya bean oil; 6.07 g. of ether insoluble solid was obtained. This solid was extracted thoroughly with hot water. The aqueous extract was allowed to cool down, and from it was obtained 1.66 g. of dried product. This dry material was subjected to fractional crystallization in hot alcohol. The solid which separated from the warm solution was found to melt at 174°. Upon evaporating the filtrate, a second crop was obtained melting at 158–161°. After reducing the volume of the filtrate, a third crop was obtained. It was a very small amount, melting at 158–160°. The two lower fractions were fractionally crystallized together. While the solution was still warm, a fraction of solid was obtained which was found to melt at 164°. A second fraction was obtained melting at 156–158° from which a small quantity of solid melting at 164° was isolated. A small portion melting at 155–158° remained. This was undoubtedly a mixture of the two sativic acids. Oxidation was repeated and the same results were obtained.

**Preparation of Linolic Acid Tetrabromide of the Free Fatty Acids of Cottonseed Oil.**—Bromination of the dry ethereal solution of 336 g. of the free fatty acids of cottonseed oil was carried out in the same manner as previously described. Crystallization of the solid tetrabromide was done in low boiling petroleum ether. The compound obtained after two recrystallizations melted at 114°. No solid tetrabromide of lower melting point was isolated.

**Regenerated Linolic Acid of Cottonseed Oil.**—This was prepared as previously described elsewhere in this paper: 34.918 g. of $\alpha$-linolic acid tetrabromide, m. p. 114°, was the starting material. Digestion on the water-bath was continued for a period of fifty-two hours. Care was exercised in the different operations to avoid loss of material; 16.885 g. of product was obtained. From these figures the percentage yield and purity of product was found to be 97.6%.

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[8] Tsujimoto [*Chem. Rev. Fett- Harz-Ind.*, **18**, 111 (1911)] reported having obtained a tetrahydroxystearic acid from soya bean oil melting at 156–157.5°. This is probably not a pure acid and undoubtedly a mixture of the two sativic acids melting, respectively, at 164 and 174°.

TABLE II

ANALYTICAL DATA[a]

| Substance | Sample used, mg. | $CO_2$, mg. | $H_2O$, mg. | %, C Found | %, C Calcd. | %, H Found | %, H Calcd. |
|---|---|---|---|---|---|---|---|
| β-Sativic acid | 5.548[b] | 12.553 | 5.074 | 61.70 | 62.1 | 10.24 | 10.3 |
|  | 5.647[c] | 13.023 | 5.280 | 62.85 | 62.1 | 10.45 | 10.3 |
| α-Sativic acid | 5.732[b] | 13.110 | 5.211 | 62.38 | 62.1 | 10.18 | 10.3 |
|  | 5.395[c] | 12.287 | 4.900 | 61.70 | 62.1 | 10.16 | 10.3 |
| α-Linolic acid tetrabromide | 5.761[d] | 7.759 | 2.753 | 36.73 | 36.02 | 5.35 | 5.34 |

[a] The writer wishes to acknowledge his indebtedness to Prof. A. C. Santos of the College of Pharmacy, University of the Philippines, for the micro-analysis.

[b] Analysis of the sativic acids obtained from linolic acid present in the oil of soya bean oil and from the regenerated linolic acid from α-linolic acid tetrabromide of this oil. The sample is a mixture of the corresponding acids obtained from the free acids of the oil and from the regenerated acid.

[c] Analysis of sativic acids from the free fatty acids of cottonseed oil and its regenerated linolic acid. Samples were made in the same manner as with soya bean.

[d] Sample was a mixture of α-linolic acid tetrabromide prepared from the free fatty acid of soya bean oil and its regenerated linolic acid and from the free fatty acids of cottonseed oil. When the sample was recrystallized from a solution of petroleum ether and ordinary ether (1:1), the melting point was raised up to 115–116°. W. C. Smit [*Rec. trav. chim.*, **49**, 539 (1930)] obtained 116–117° as the melting point of the same compound.

**Oxidation of the Regenerated Linolic Acid[9] from α-Linolic Acid Tetrabromide of Cottonseed Oil.**—5.198 grams of the regenerated linolic acid was oxidized with cold alkaline permanganate solution in the same manner as already described; 3.026 g. of product was obtained. The dried product was washed with ether and then extracted with hot water. The extracted fraction amounted to 1.874 g., m. p. 156–158°. It was washed with hot alcohol. The first fraction of solid obtained while the solution was still warm was found to melt at 174°, second fraction at 156–158°, third fraction at 156–158°. The lower fraction was fractionally recrystallized from hot alcohol. The first crop was found to melt at 163–164°, second crop at 163–164°, third crop at 156–159°. The third crop was undoubtedly a mixture of the two sativic acids. Oxidation was repeated and the same results were obtained.

The purity of the compounds here reported was checked by micro-analysis.

## Summary

1. Only one solid tetrabromide, α-linolic acid tetrabromide (m. p. 114°), is obtainable from the direct bromination of the free fatty acids of soya bean and cottonseed oils.

2. Only one solid tetrabromide, α-linolic acid tetrabromide (m. p. 114°), is obtainable from the bromination of the regenerated linolic acid from the α-linolic acid tetrabromide (m. p. 114°) of soya bean oil.

3. Only two tetrahydroxystearic acids, α-sativic acid (m. p. 163°) and β-sativic acid (m. p. 174°) are obtainable from the free fatty acids of soya bean and of cottonseed oils.

4. Only α-sativic acid (m. p. 164°) and β-sativic acid (m. p. 174°) are obtainable from the oxidation with cold alkaline permanganate solution of the regenerated linolic acid from the α-linolic acid tetrabromide prepared from soya bean and cottonseed oils.

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(9) Nicolet and Cox[2] reported their β-sativic acid as melting at 170° and their α-sativic acid as melting at 153°. Their acids melted appreciably much lower and may still be impure. Meyer and Beer's[2] observation which was doubted by Nicolet and Cox finds strong support in the result of the oxidation of linolic acid actually present in cottonseed oil and of the regenerated linolic acid. S. R. Benson and H. N. Calderwood [THIS JOURNAL, **58**, 523 (1936)] have reported two tetrahydroxystearic acids, m. p. 152 and 173°, which they considered identical to the compounds of Nicolet and Cox. T. G. Green and T. P. Hilditch [*Biochem. J.*, **29**, 1552 (1935)] reported two solid tetrahydroxystearic acids melting at 155 and 173°.