The product was identified as succinic acid by a mixed melting point determination with an authentic specimen (m. p. 189°).

The solution of steam-volatile acids was neutralized with sodium hydroxide solution and evaporated to dryness on the steam-bath, and the residue was converted to the *p*-phenylphenacyl ester, melting point and mixed melting point with an authentic sample of *p*-phenylphenacyl butyrate, 81–82°.

Exposure of VIII to Ultraviolet Light.—One gram of compound VIII was exposed on a watch glass for eighteen hours to the direct light from an ultraviolet lamp. The resulting orange viscous oil was taken up in a few milliliters of Skellysolve A, in which a very small quantity remained insoluble, and cooled in Dry Ice–acetone mixture. The mass of colorless needles that appeared weighed 0.9 g. (90%) after filtration and drying. A melting point and mixed melting point of 54–55° showed the material to be unchanged VIII.

The 100 mg. of orange resinous material insoluble in Skellysolve A could not be made to crystallize.

Summary

A stable, geometrical isomer of natural pellitorine (N-isobutyl-2,6-decadienamide) has been synthesized. In contrast to the natural isomer, it is non-pungent and non-toxic to house flies.

Hydrogenation of the amide gave N-isobutylcapramide, and on oxidation it yielded butyric, succinic, and N-isobutyloxamic acids.

Exposure to ultraviolet light failed to transform the synthetic into the natural isomer.

BELTSVILLE, MD. RECEIVED SEPTEMBER 28, 1949

[CONTRIBUTION FROM THE CHEMICAL DEPARTMENT AT THE FACULDADE DE FILOSOFIA, CIÊNCIAS E LETRAS DA UNIVERSIDADE DE SÃO PAULO]

Some Constituents of the Leaves of *Cassia alata L.*[1]

BY H. HAUPTMANN AND L. LACERDA NAZÁRIÔ

Cassia alata L., one of the 189 Brazilian *Cassia* species, is in its pharmacological action similar to senna leaves (*Cassia angustifolia Vahl* and *Cassia acutifolia Delile*) as Wasicky[2] pointed out some years ago. At the same time, based on the Borntraeger reaction,[3] the same author came to the conclusion that there are anthraquinone derivatives in reduced state either free or in glycosidal combination. Earlier studies on this plant[4] mention only hydroxymethylanthraquinones or "chrysophanic acid" as isolated compounds. Therefore, it seemed interesting to try the isolation and identification of its main constituents.

Cassia alata leaves were extracted with 25% alcohol[5] after a previous treatment with petroleum ether.[6] The alcoholic extract was submitted to two different treatments: (a) fractional precipitation with lead acetate similar to that used by several investigators[7] in their studies on senna leaves and (b) hydrolysis with sodium carbonate.

By the first procedure (a) all the anthraquinone compounds and their reduced derivatives were precipitated when an excess of saturated lead acetate solution was added to the ethanolic extract at room temperature. Heating should be avoided because at higher temperature the precipitation is not quantitative. By fractionating the lead salt precipitate different fractions A were obtained which all contained anthraquinonic substances either in reduced or in oxidized state. The first fraction (A1) obtained by extraction of the lead salt precipitate with alcohol was microcrystalline and showed a positive Borntraeger reaction only after several hours. It was extremely sensitive to air, darkened and liquefied within a few seconds when exposed. The dark oil gave immediately a positive Borntraeger reaction. This behavior leads us to the conclusion that the micro-crystalline fraction contained reduced anthraquinones which, on contact with the air, were very rapidly oxidized to anthraquinones.

It was impossible to isolate the anthraquinones formed by air oxidation; but oxidizing the microcrystalline precipitate of reduced anthraquinones with ferric chloride we obtained Rhein (1,8-dihydroxyanthraquinone-3-carboxylic acid, m. p. 310°) which was identified by direct comparison with an authentic sample. Further identification involved preparation of its acetate (m. p. 217–218°), methyl ester (m. p. 172–174°), methyl ester acetate[8] (m. p. 194–195°). The purification of Rhein was made difficult by the presence of very small quantities of another anthraquinone that, up to now, could not be identified. From the lead salt precipitate after decomposition with hydrogen sulfide two more fractions (A2 and A3) could be obtained by extraction with benzene and alcohol, respectively. A2 yielded Rhein directly without previous oxidation. From A3, which

(1) Extract from a Thesis presented by L. Lacerda Nazárió to the Faculdade de Filosofia, Ciências e Letras da Universidade de São Paulo, Brasil, for the degree "Doutor em Ciências."

(2) R. Wasicky, *Anias. Fac. Farm. Odont. Univ. S. Paulo*, **2**, 57 (1942).

(3) H. Borntraeger, *Z. anal. Chem.*, **19**, 165 (1880).

(4) Porte and Helbing, *Z. Österr. Apoth. Ver.*, 589 (1884), cited after C. Wehmer "Pflanzenstoffe," G. Fischer, Jena, 1929, p. 505; Gresshof, *Ber.*, **23**, 3540 (1890).

(5) Private communication of R. Wasicky.

(6) P. B. Murti and T. R. Seshadri, *Proc. Indian Acad. Sci.*, **10A**, 96 (1939).

(7) A. Tschirch, *Schweiz. Wochenschr. Pharm.*, **23**, 174 (1898); *Ber. deuts. Pharm. Ges.*, **8**, 174 (1898).

(8) This compound was found to be especially satisfactory for identification purposes (private communication of the Scientific Laboratory of Sandoz A. G., Basel).

contained reduced anthraquinones, Rhein could be isolated after oxidation with ferric chloride.

The melting points indicated for Rhein vary between 312° and 326–329°.[9a,b] When using the Kofler hot-plate, which permits a good verification of the melting point even with dark and decomposing substances, we always observed melting at 310°, and when heated slowly enough, the substance melted entirely at that temperature. With a capillary no sharp melting point could be established. The substance melted in an interval with decomposition beginning at about 310°. The indicated melting points of Rhein acetate are between 240 and 258°.[9c,d] In our hands neither the Rhein acetate prepared from the authentic Rhein nor that isolated from the plant had a melting point higher than 218°. It seems possible that we obtained another modification. In spite of these differences, there cannot be any reasonable doubt about the identity of our substances when one considers not only the excellent analysis of Rhein and Rhein acetate but also the properties of the other two derivatives prepared and the results of the mixed melting points with the authentic samples.

The Molisch and Fehling reactions indicated the presence of sugars in the lead salt precipitate. From the anthraquinone-free filtrate two osazones were isolated, rhamnose phenylosazone and glucose phenylosazone. Although this compound can arise from glucose, mannose or fructose it is probably from glucose which is most frequently found in *Cassia* plants.

The hydrolysis of the ethanolic extract with sodium carbonate (the second procedure (b) employed) yielded a yellow crystalline substance that melted with decomposition at 335–336°. Its formula is $C_{15}H_8O_7$. When treated with diazomethane it yielded a dimethyl ester $C_{17}H_{12}O_7$ (m. p. 186–187°). We have been unable to obtain this yellow substance by other procedures such as direct extraction, acidic hydrolysis of the plant extract or lead acetate precipitation. Therefore, it seems not to exist originally in the leaves but to be formed during the alkaline hydrolysis. We observed its formation when boiling a fraction of reduced anthraquinones with sodium carbonate solution. Perhaps it is identical with the "pale yellow crystals" which M. Anchel[9d] isolated after extracting *Cassia reticulata* with sodium carbonate. If the substance is really produced by alkaline degradation of those compounds, one ring has been opened. This is easily seen from the fact that a dicarboxylic acid with an unmodified anthracene structure has at least sixteen carbon atoms. Beside this compound, the alkaline hydrolysis yielded anthraquinone and reduced anthraquinone derivatives accompanied by phytosterols, resinous and fat-like substances.

(9) (a) O. Hesse, *J. prakt. Chem.*, [2] **77**, 388 (1908); (b) O. A. Oesterle and E. Tisza, *Schweiz. Wochenschr. f. Chemie u. Pharmacie*, **46**, 202 (1908); (c) F. Tutin, *J. Chem. Soc.*, **103**, 2006 (1913); (d) M. Anchel, *J. Biol. Chem.*, **177**, 169 (1949).

The best way to separate these complex mixtures seemed to be a treatment with appropriate adsorbents. In previous experiments using chromatographic methods we (as well as Gibson and Schwarting)[10] were unable to reproduce the results of Ernst and Weiner.[11] In agreement with the results of Gibson and Schwarting and Tumminkatti and Beal,[12] we found that on magnesium oxide, alumina or charcoal, the anthraquinone compounds were irreversibly adsorbed and could not be eluted, while silica was too weak and did not adsorb them. We found Magnesol[12a] to be an appropriate adsorbent which allowed the separation of the anthraquinone derivatives. Here, too, Rhein proved to be the main anthraquinone derivative accompanied by small quantities of other unidentified anthraquinonic substances.

The isolation of Rhein demonstrates that *Cassia alata* is, in fact, closely related to the *Cassia* species of senna leaves not only because of its cathartic action but also because of the chemical character of its main component. A considerable portion of the anthraquinone compounds, especially of Rhein, is present in reduced state in the leaves of *Cassia alata*. This represents one more analogy to senna leaves from which Stoll[13] isolated glycosides of reduced anthraquinone compounds in crystalline state and proved that they were responsible for the cathartic action. The crystallization of the aglycones of these glycosides seems to be very difficult as indicated by the reports of Stoll, *et al.*, even when the pure crystalline glycosides were hydrolyzed. Their proof is the same as ours (*i. e.*, isolation of Rhein after oxidation). Recently M. Anchel[9d] identified the antibiotic substance of *Cassia reticulata* as being Rhein. Rhein and its derivatives have been hitherto considered as being without physiological or pharmacological importance. In view of the findings of Stoll, Anchel and ourselves, this opinion must be revised.

Experimental

All m. p. were taken with the Kofler apparatus. Analyses executed by Arlington Laboratories, Fairfax, Virginia, and Dr. F. Weiser, Basel, Maulbeerstr. 41.

Lead Salt Precipitation.—Three hundred grams of *Cassia alata* leaves was extracted first with Benzo-sol[13a] and then with 25% alcohol. To the cold alcoholic extract, a cold aqueous solution of saturated lead acetate was added until no further precipitate was formed. After being cooled in the refrigerator for six hours the dark brown precipitate (A) was filtered. To the filtrate some drops of ammonium hydroxide were added. The yellow pre-

(10) M. Gibson and A. Schwarting, *J. Am. Pharm. Assoc.*, **37**, 206 (1948).

(11) P. Ernst and G. Weiner, *Sci. Pharm.*, **8**, 45 (1937), cited after L. Zechmeister and L. Cholnoky, "Principles and Practice of Chromatography," Chapman and Hall Ltd., London, 1941, p. 296.

(12) M. Tumminkatti and G. D. Beal, *J Am. Pharm. Assoc.*, **15**, 847 (1926).

(12a) Magnesol, product of the Westvaco Chlorine Products Corp., Newark, Calif.

(13) A. Stoll, W. Kussmaul and B. Becker, *Verhandl. naturforsch. Ges. Basel*, 235 (1941); Sandoz, Ltd., British Patent 555,450 (1943); *C. A.*, **39**, 783 (1945).

(13a) Available from the Shell Petroleum Company.

cipitate (B) formed was separated by filtration and the colorless filtrate (C) was concentrated under reduced pressure. The dark brown precipitate (A) when decomposed with sulfuric acid gave a positive Borntraeger reaction only after several hours of exposure to air. The yellow precipitate (B) gave an intense yellow color with ammonia and a dark green precipitate with ferric chloride, and the filtrate (C) a positive Fehling test for sugars.

Precipitate A.—It was exhaustively extracted with hot alcohol. The hot alcoholic extract (A1) was evaporated to dryness in a carbon dioxide current. A brown powder was obtained (2.87 g.) which, when exposed to air, was immediately transformed into a dark oil. It gave no Fehling test. Oxidized with ferric chloride[9] it yielded 35 mg. of Rhein (m. p. 308–310°).

The lead precipitate (A), after being extracted with alcohol, was suspended in hot benzene and decomposed with hydrogen sulfide. The sulfide precipitate was filtered off from the benzene solution (A2), then refluxed with alcohol (A3) and discarded.

A2.—After evaporation to dryness the residue of the yellow benzene solution was treated with sodium carbonate solution to separate the anthraquinones from the sulfur present. The alkaline solution was acidified and extracted with ether. The ethereal solution was washed with a saturated sodium chloride solution and then concentrated in the presence of a drop of pyridine. Eight milligrams of Rhein crystallized from the pyridine.

A3. Alcoholic Solution.—Evaporated to dryness (5 g.) it showed almost the same characteristics as the A1 precipitate, especially the same sensitivity to air. Oxidized with ferric chloride[9] 3 g. gave 39.5 mg. of Rhein (m. p. 308–310°) and a very small portion of another anthraquinone (m. p. 250–260°). This fraction gave also a positive Molisch test for sugars.

Precipitate B (Yellow Precipitate).—It was suspended in water and decomposed with hydrogen sulfide and extracted with boiling water. This solution was concentrated and then hydrolyzed with 7% sulfuric acid. After extraction with ether to separate a yellow substance, probably a flavone, phenylhydrazine hydrochloride and sodium acetate were added to the neutralized aqueous solution. The mixture of phenylosazones found was treated with acetone. The acetone soluble fraction was recrystallized from 70% alcohol in presence of charcoal and yielded a glucose phenylosazone of m. p. 203–205°. The acetone insoluble fraction was extracted with benzene and then crystallized from 50% alcohol. The crystalline product melted at 183–185° and was found to be identical with rhamnose phenylosazone, showing no depression of the mixed melting point with an authentic sample.

Filtrate C.—This fraction was freed from the excess of lead ions with hydrogen sulfide. After filtering, it was heated with 2 g. of phenylhydrazine hydrochloride and 4 g. of sodium acetate dissolved in 60 ml. of water, and the resulting brown oily precipitate washed with hot benzene and recrystallized from 50% alcohol in the presence of charcoal. Rhamnose phenylosazone, melting at 182–184°, was obtained and identified by the mixed melting point, 182–184°, with an authentic sample.

Sodium Carbonate Hydrolysis.—Six hundred and fifty grams of powdered leaves of *Cassia alata* were extracted repeatedly with Benzo-sol in a large continuous extractor until further extraction gave a colorless solution. The leaf-powder was completely freed from the last traces of solvent by passing air through it. It was then exhaustively extracted with 25% alcohol. The dark colored extract was concentrated to two-thirds of the original volume under reduced pressure in a carbon dioxide current. To the hot concentrated extract sodium carbonate was added in such an amount as to give a 3% solution and then boiled for one hour. After cooling it was extracted with ether. The ether extract which gave a positive Liebermann-Burchard test was discarded. The alkaline solution was then acidified with hydrochloric acid and again extracted with ether in a large continuous liquid extractor. The ether extract (Borntraeger test positive) was evaporated to dryness in a carbon dioxide current. When the residue was treated with acetone, a yellow substance was crystallized in a yield of 0.24%. It gave no Borntraeger test. After recrystallization from acetone it melted at 335–336°.[14]

Anal. Calcd.: C, 59.98; H, 2.68. Found: C, 60.10; H, 2.82.

Dimethyl Ester.—By standing for fourteen hours with diazomethane in methanolic solution, extraction of the residue with petroleum ether (b. p. 50–70°) and three recrystallizations from methanol in presence of charcoal, a yellow substance was obtained in 60.5% yield, m. p. 186–187°.

Anal. Calcd.: C, 62.18; H, 3.62; mol. wt., 328; sapon. equiv., 164. Found: C, 62.23; H, 3.63; mol. wt., 318; sapon. equiv., 160.9.

The mother liquors were evaporated to dryness. The residue (1.08% of the leaves) was then mixed with sand and extracted with benzene (I), ether (II) and acetone (III).

I. **Benzene Extract.**—To the benzene solution, Magnesol was added until it was no longer colored (orange red to yellow). Then the adsorbent was filtered and exhaustively extracted with hot benzene, ether, alcohol, water and finally diluted (5%) hydrochloric acid and ether; then it was discarded.

Only the acidic ether extract contained anthraquinone substances. It was extracted with 5% sodium carbonate and this solution was acidified and extracted with ether. The residue of this ethereal solution was recrystallized from pyridine and yielded 22 mg. of Rhein of m. p. 308–310°.

II. **Ether Extract.**—The ether extract gave a positive Borntraeger reaction only after several hours. It was evaporated to dryness and the residue treated with chloroform. The insoluble part which gave a positive Borntraeger reaction after several hours was treated with aqueous alcohol. The resulting micro-crystalline product melted between 140 and 150°. In spite of a concerted effort no further purification was possible. Therefore, the substance was oxidized with chromic acid.[15] The immediate positive Borntraeger reaction showed that anthraquinonic substance had been formed, but it could not be obtained in pure state.

III. **Acetone Extract.**—Additional 100 mg. of the dicarboxylic acid of m. p. 335–336° could be isolated by evaporation to dryness and recrystallization as described above.

Identification of Rhein.—Rhein was purified by crystallization from pyridine and sublimation in high vacuum. Then it melted at 309–310° (dec.). The mixed melting point with an authentic sample of m. p. 308–310° showed no depression.

Anal. Calcd.: C, 63.36; H, 2.83; 3 active H, 1.11. Found: C, 63.25; H, 3.07; active H, 1.06.

Acetyl Rhein.—One hundred and two milligrams of Rhein, 2 ml. of acetic anhydride and two drops of concentrated sulfuric acid were boiled for one-half hour. After cooling, water was added, the precipitate filtered and crystallized from alcohol and glacial acetic acid; yield 96.6 mg. of m. p. 217–218° (dec.); mixed m. p. with acetate prepared from authentic Rhein, 216–218°.

Anal. Calcd.: C, 61.95; H, 3.28. Found: C, 62.05; H, 3,58.

Methyl Ester of Rhein.—This substance was prepared as described by Robinson and Simonsen.[15] An orange substance of m. p. 172–174° was obtained.

Anal. Calcd.: C, 64.41; H, 3.31. Found: C, 64.61; H, 3.46.

Methyldiacetyl Rhein.—Eighty-six milligrams of diacetyl Rhein was dissolved in a mixture of ether and methanol and treated with 10 ml. of an ether solution of diazomethane recently prepared. After fourteen hours, the ether was evaporated and a few drops of glacial acetic

(14) See M. Anchel, THIS JOURNAL, **72**, April (1950).

(15) R. Robinson and J. L. Simonsen, *J. Chem. Soc.*, **95**, 1085 (1909).

acid added. The yellow crystals obtained had a m. p. of 194–195°; yield 50 mg.; mixed m. p. with diacetyl Rhein methyl ester prepared from authentic Rhein, 194–195°.

Acknowledgments.—Prof. Dr. R. Wasicky directed our attention to this problem. Prof. Dr. A. Stoll (Sandoz A. G., Basel, Switzerland) kindly supplied authentic Rhein. The Rockefeller Foundation supported this work by means of a grant. We wish to express our thanks to all.

Summary

Hydrolysis of extracts from *Cassia alata* leaves yields Rhein which occurs in the plant mainly in a reduced state as glycosides. Rhamnose and glucose were isolated as their phenylosazones.

SÃO PAULO, BRASIL RECEIVED AUGUST 4, 1949

[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF MCGILL UNIVERSITY AND THE UNIVERSITY OF TORONTO]

The Extraction of Birch Lignins with Acetic Acid[1]

BY ALAN BELL AND GEORGE F WRIGHT

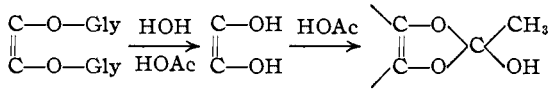
Comparison of the extracted lignins from various woods, and especially those from hard and soft woods, is of value in the elucidation of the structure of lignins. Since the extraction process always seems to alter the lignin from its original state in the wood, it is also useful to compare the lignins variously extracted from a certain wood. The extraction of birch wood with formic acid has already been reported.[2] Acetic acid has been used in the present investigation, and the extracted lignin separated into ether-soluble, benzene-soluble, methanol-soluble and chloroform-soluble fractions.

The expectation that lignins would be more easily extracted from a hard than from a soft wood, such as spruce, has been realized. The birch lignin was found to be extracted slowly in boiling glacial acetic acid over a four-day period. The products were notably more soluble in organic solvents than any heretofore obtained. This is owing, in part, to the presence of acetyl groups which apparently protect the lignins from undue decomposition during extraction. Moreover under completely anhydrous extraction conditions the least soluble part of the extract is found to contain 10–20% of carbohydrate chemically attached to the lignin. The isolation of this lignin-carbohydrate complex confirms previous conjectures concerning its existence.[3]

Most of the acetylation which occurs during extraction of birch lignin with acetic acid must take place on the attached carbohydrate. Such acetylation by boiling acetic acid (complete with monosaccharides and partial with polysaccharides) is not unknown.[4,5] The position of the acetyl groups is indicated by the elimination of about 7–10% of this group (compare Tables I and II) together with combined carbohydrate when the lignin fractions obtained by acetic acid extraction subsequently are digested with formic acid. This acid, stronger than acetic, would not be expected to cause de-acetylation (except perhaps by ester interchange) but it would tend toward glycoside hydrolysis. It would be especially effective in this action if the glycoside linkage were phenolic.

The acetic acid-extracted lignin fractions (hereafter called acetic lignins) have for convenience been designated as acetic-formic lignins after they have been digested with formic acid. The residual 7% of acetyl which remains after such digestion conforms with about one ester group per kilogram. This is approximately equivalent to the number of formyl groups which are found by analysis of birch lignins directly extracted by formic acid.[2]

These residual ester linkages seem unreactive toward Grignard reagent. Thus a lignin recovered from Grignard analysis of a sample containing 16% acetyl (1.2 RMgI per $COCH_3$ in dioxane) was found still to contain 9% of acetyl which would not react with the Grignard reagent. Since acetyl groups are not present in the original lignin as it exists in the wood, they evidently are introduced during the extraction process. Part of this acetylation must take place on the chemically bound carbohydrate, but the residual (and inert) acetyl seems to be present in the non-carbohydrate portion. A possible explanation would, in part, represent the lignin extraction process as



I II III

where Gly in I typifies any glycoside. The acetylation of an intermediate ene-diol, II, to form a 2-methyl-2-hydroxydioxole, III would explain the "hidden" hydroxyl groups which are suspected in fatty acid-extracted lignins.[2]

Not all of the glycosidically bound hydroxyl groups in lignin can, however, be explained in this way. Other acidic hydroxyl groups must be present. These have been demonstrated by "com-

(1) The authors are grateful for aid from the National Research Council of Canada and from the Canadian Pulp and Paper Association. They wish also to thank Dr. Harold Hibbert.

(2) M. Lieff, G. F. Wright and H. Hibbert, THIS JOURNAL, **61**, 1477 (1939).

(3) (a) E. E. Harris, E. C. Sherrard and R. L. Mitchell, *ibid.*, **56**, 889 (1934); (b) A. G. Norman and J. G. Shrikhande, *Biochem. J.*, **29**, 2259 (1935); (c) H. Hibbert and W. H. Steeves, THIS JOURNAL, **59**, 1768 (1937).

(4) C. J. Malm and H. T. Clarke, *ibid.*, **51**, 274 (1929).

(5) H. T. Clarke and H. B. Gillespie, *ibid.*, **54**, 2083 (1932).