and then poured onto ice. The crude semisolid was treated with 50–100 ml of hot ligroin and filtered. This solution afforded 0.3 g of recovered starting material and 0.5 g of crude quinone **6**, mp 111–115°; the infrared spectrum was identical with that of a known sample. The ligroin-insoluble material was recrystallized first from a large volume of ligroin and then from ethanol–water to afford 0.3 g of pale yellow product **5**: mp 153–155° (sinter 145°); ν_{max}^{KBr} 3420 (-OH) and 1660 cm^{-1} (conj C=O); $\lambda_{max}^{C_2H_5OH}$ 304 mμ (log ε 3.66); one olefinic proton ($CDCl_3$) at τ 3.14 ppm (singlet).

Anal. Calcd for $C_{18}H_{16}O_3$: C, 77.1; H, 5.8. Found: C, 77.5; H, 5.9.

Registry No.—**3**, 14908-33-9; acetate of **3**, 15038-92-3; **5**, 14908-34-0; **8**, 14908-35-1; **9**, 14908-36-2; 2-acetoxy-5-phenyl-1,4-benzoquinone, 14908-37-3.

Constituents of *Brucea sumatrana* Roxb. I. Brusatol[1]

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Received August 3, 1967

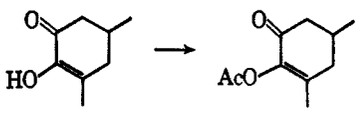
Recent studies on the bitter principles of a number of genera of the family Simaroubaceae have brought to light a group of structurally allied compounds with close chemical and botanical relationships with each other and other constituents of plants of the families Meliaceae and Rutaceae.[3] A number of the simaroubaceous principles are well known in herbal medicine as effective antiamebic agents.[4] A genus of this family that is widely known in Asia for its antidysenteric properties and whose chemical constitution has come under occasional study[5] is *Brucea*, of which *B. sumatrana*, *B. javanica*, *B. antidysenterica*, and *B. amarissima* are known under various local names (ko-sam, ya-tan-tzu, k'u-shen-tzu, lao-ya-tan) as herbal remedies for human amebiasis. Earlier chemical studies[5] resulted in the isolation of a number of crystalline compounds, none of which, however, was well characterized.

We have examined the seeds of *Brucea sumatrana*[6] and have isolated a crystalline compound, brusatol.[7] Brusatol, mp 276–278°, is very bitter, gives a dark green color with ferric chloride, and no color with concentrated sulfuric acid.[8] It could be acetylated to yield a monoacetate and a triacetate, in the first of which the loss of the ferric chloride color reaction and the shift in the ultraviolet absorption spectrum from that characteristic of a diosphenol to one showing the characteristics of an α,β-unsaturated ketone indicated that brusatol was a diosphenol that contained two additional hydroxyl groups and that the diosphenolic hydroxyl group was the first to undergo acylation.

Elementary and functional group analyses of brusatol showed that it has the composition $C_{26}H_{32}O_{11}$ and contains a methoxyl group and what was at first thought to be an acetyl group.[7,9] Early analytical results of acetyl group and methoxyl group determination were not mutually consistent with any molecular formula. The reason for this became clear when brusatol was at length found to be a senecioyl and not an acetyl ester.

The mass spectrum of brusatol showed a weak molecular ion at *m/e* 520, which corresponds to the formula $C_{26}H_{32}O_{11}$, a peak at *m/e* 502 (M − H_2O), and a base peak at *m/e* 55. A peak at *m/e* 83 in brusatol monoacetate corresponds with the fragment $((CH_3)_2C{=}CHCO)^+$, a conclusion that is supported by the observation that in the mass spectrum of dihydrobrusatol are observed peaks at *m/e* values of 57 and 85. These values further suggest that the values *m/e* 55 and 57 are due, respectively, to the ions $((CH_3)_2C{=}CH)^+$ and $((CH_3)CHCH_2)^+$ (or $(CH_3)_3C^+$), formed by the loss of carbon monoxide from the corresponding acylium ions.

The ultraviolet spectrum of brusatol gave a clear indication that a diosphenolic grouping was present. Brusatol has λ_{max} 219 mμ (log ε 4.13) and 279 (3.88) in ethanol and upon addition of alkali the lower wavelength peak remains unaltered while the other shifts to 320 mμ. The brusatol acetates retain the peak at about 220 mμ, but the higher wavelength peak of brusatol is replaced by a shoulder at about 240 mμ (log ε about 4). These observations, along with the nmr spectra, which show the vinylic methyl group but no vinylic hydrogen, are in accord with the presence in brusatol of the grouping **1**, converted by acetylation into **2**.



1 2

The nmr spectra of brusatol (**3**) (measured in pyridine) and its two acetyl derivatives provide information which, along with the assumption that the compound is one of the group of simarolides characteristic of the plant family, define most of the structural features of the molecule. In particular, three-proton singlets at δ 1.90 and 2.11 (in brusatol), the latter of which was initially assigned[7] to an acetyl group, disclosed the presence of the senecioyl grouping, and signals for two protons at δ 4.23 in the monoacetate which appeared at δ 5.32 in the triacetate showed that brusatol contained two secondary hydroxyl groups. A three-proton singlet at δ 3.78 (in the monoacetate) and δ 3.72 (in the triacetate), which disappeared upon alkaline hydrolysis, indicated the presence of a methoxycarbonyl grouping.

(1) Contribution No. 2124 from the Department of Chemistry, U.C.L.A.

(2) University of Singapore; Fulbright Research Scholar, 1967, University of California, Los Angeles.

(3) For a recent review of the literature of studies on simaroubaceous plants, see J. Polonsky, *Planta Med. Suppl.*, 107 (1966).

(4) T. A. Geissman, *Ann. Rev. Pharmacol.*, **4**, 305 (1964).

(5) Y. T. Chang, *Chinese Med. J.*, **69**, 87 (1951).

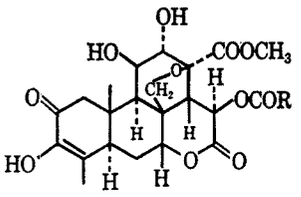
(6) The seed was procured by S. B. Penick and Co. We are grateful to Mr. Hans R. Schmidt, pharmacognosist, for his efforts in procuring the material and authenticating its identification and in providing us with the crude extracts.

(7) A preliminary account of this work was presented at the colloquium on Phytochemistry and Medicinal Plants of the Pacific Area, Nouméa, New Caledonia, May 1964, and appears in *Bull. Groupe Franc. Argiles*, **144**, 205 (1966).

(8) A characteristic property of a number of the bitter lactones found in the simaroubaceous plants is the intense red to blue colorations that they show when treated with concentrated sulfuric acid.

(9) Brusatol was originally reported[7] to have the composition $C_{25}H_{44}O_{11}$. Mass spectra later provided evidence which made possible the revision to the composition now known to be correct.

These and other nmr data thus showed the presence in brusatol of many of the structural features that are known to be present in the numerous lactones that have been isolated from various simarubaceous plants[3] and made it possible to accept the hypothesis that brusatol possessed the structure **3**.[10]



3, R = CH=C(CH$_3$)$_2$
4, R = CH$_2$CH(CH$_3$)$_2$

The presence of the senecioyl grouping was demonstrated by the preparation of dihydrobrusatol (**4**). The dihydro compound, $C_{26}H_{34}O_{11}$, showed a small molecular ion peak of *m/e* 522 in the mass spectrum and in addition showed a base peak of *m/e* 57 and a prominent peak of *m/e* 85. The compound still gave a green ferric chloride color and had λ_{max} 281 mμ, showing that the diosphenol structure was still present. It gave a mono- and a triacetate (the first of which was not crystalline but was homogeneous on a thin layer chromatogram), neither of which gave a ferric reaction. The nmr spectrum of dihydrobrusatol still contained the three-proton singlets for the quaternary methyl group (δ 1.57) and for the vinylic methyl group of the diosphenolic grouping (δ 1.91) but lacked the signals for the methyl groups of the senecioyl grouping. In their place appeared a new six-proton signal at high field (δ 0.94, doublet, J = 6 cps). Further, the one-proton signal for the vinylic proton at δ 5.66 (in the monoacetate) and δ 5.63 (in the triacetate) was no longer present in the spectra of the dihydro compounds. These observations pointed clearly to the presence in brusatol of the senecioyl grouping, altered to the isovaleryl grouping by hydrogenation.

That brusatol is indeed an O-senecioyl ester was finally established by saponification and isolation of senecioic acid, identical with authentic material.

At this point in our studies we became aware of the work of Polonsky on the bitter principles of *Brucea amarissima*.[11] The three compounds isolated from *B. amarissima*, called bruceines A, B, and C, were different from brusatol, but bruceine A was assigned the structure of dihydrobrusatol.[11] A direct comparison (melting point and mixture melting point, optical rotation, thin layer chromatography, and infrared spectrum) showed the identity of dihydrobrusatol with bruceine A. The conclusions of Polonsky, *et al.*, regarding other features of the structure of bruceine A are in complete accord with our interpretations of our data and, along with the compelling weight of evidence regarding the biosynthetic interrelationships in the lactones of this and related groups,[3] leave little doubt of the correctness not only of the gross structures of **3** and **4** but of their stereochemistry as well.

A study of the mixtures remaining after separation of brusatol from the *Brucea* extracts disclosed the presence of small amounts of compounds showing slightly different mobility on thin layer chromatograms than brusatol, but the latter is the major component of the fraction from which it was obtained. However, the presence of several nonlipophilic substances in the aqueous syrup from which brusatol was extracted is shown by the appearance on thin layer chromatograms of the aqueous solution of three distinct components that give intense purple colors upon spraying with concentrated sulfuric acid.[8] Attempts to isolate these compounds are in progress.

(10) Although the disposition of all of the groups shown in **3** was not at first unambiguously defined by the nmr data, **3** is shown as the structure now known to be correct.

(11) J. Polonsky, Z. Baskevitch, A. Gaudemer, and B. C. Das, *Experientia*, in press. We are grateful to Mme. Polonsky for a copy of the galley proof of her article prior to its publication.

Experimental Section

Melting points were determined in capillary tubes with a Swissco melting point apparatus and are corrected. Ultraviolet spectra were measured in 95% ethanol on a Cary Model 14 spectrophotometer and infrared spectra were taken in KBr disks with a Perkin-Elmer Model 237 spectrophotometer. Nmr spectra, unless otherwise specified, were determined in deuteriochloroform solution, with tetramethylsilane as an internal standard, with a Varian A-60 spectrometer. Mass spectra were taken with an A.E.I. MS-9 instrument at 70 ev using direct insertion. Thin layer chromatography (tlc) was carried out with the use of silica gel G and chloroform–methanol (100:7) as the solvent.

Brusatol.—The total methanol extract of the powdered and defatted (with hexane) seeds of *Brucea sumatrana* was evaporated to a viscous residue which was dark green in color and extremely bitter and gave a brown-purple coloration with concentrated sulfuric acid. The syrupy material was mixed with an equal volume of water and the filtered solution extracted with hexane (A) until the extracts were colorless, then with methylene chloride (B).

The hexane extract (A) was dried and evaporated to a residue which did not yield crystalline material and was not examined further. The methylene chloride extract (B) was dried and evaporated to yield a dark yellow resinous material (37.8 g from 1500 g of crude methanol extract). Thin layer chromatograms of the crude material showed several components that gave green colors with a ferric chloride spray but no coloration with concentrated sulfuric acid.

The crude extract was dissolved in methanol and when the solution was allowed to evaporate slowly a crystalline material was deposited. This had mp 254–256° and weighed 6 g. Several recrystallizations from ethanol afforded material that had mp about 260–267° and still showed a trace of a contaminant on tlc: ultraviolet absorption, mμ (log ϵ), 219 (4.13), 279 (3.88); $[\alpha]^{26}D$ 43.6° (*c* 1.5, acetone).

Anal. Found: C, 60.02, 59.94; H, 6.17, 6.64; methoxyl, 8.17; O-acyl (calcd as O-acetyl), 7.87; C-methyl groups, 2.12.

Further recrystallization of the material with mp 260–267° raised this to 268–272° but failed to remove a persistent impurity (tlc). All of the specimens of mp 254–256° and higher gave infrared spectra that were identical with each other and with the material purified by chromatography (see below).

A sample of 0.33 g of brusatol was absorbed on 20 g (2.5 × 16 cm) of silica gel and eluted first with benzene, then with chloroform (100-ml fractions). Fractions 1–5 of the chloroform eluate afforded 30 mg of material which crystallized from acetone as colorless prisms, mp 273–276°.

Anal. Found: C, 60.06; H, 6.35.

Fractions 6–8 gave material that was crystallized from acetone as colorless prisms (100 mg), mp 276–278°.

Anal. Calcd for $C_{26}H_{32}O_{11}$: C, 60.00; H, 6.20; mol wt, 520. Found: C, 60.25; H, 6.33.

The tlc of the crystalline compounds from fractions 1–5 and 6–8 were identical and showed but one spot (ferric chloride spray).

The mass spectrum of the pure material showed a weak molecular ion peak at m/e 520, a weak peak at m/e 502 ($M - 18$), prominent peaks at m/e 438, 420, 402, 392, 297 (and others at lower values), and a base peak at m/e 55. The peaks at 420 and 402 correspond with loss of the molecule of senecioic acid (mol wt, 100) from brusatol and from an anhydrobrusatol, respectively.

The infrared spectrum showed prominent bands at 1745 and 1739 cm^{-1} (saturated ester and δ-lactone), a weaker shoulder at 1725 cm^{-1} (α,β-unsaturated ester), a strong peak at 1685 cm^{-1} (α,β-unsaturated ketone of the diosphenol grouping), and a peak of medium intensity at 1640 cm^{-1} (carbon–carbon double bond).

Fractions after 9 gave material that was still substantially only brusatol but which were progressively more contaminated with oily impurities.

Brusatol Monoacetate.—A solution of 92 mg of brusatol in a mixture of 1.5 ml of pyridine and 1.5 ml of acetic anhydride was kept at room temperature for 1 hr and then poured into ice-cold, dilute hydrochloric acid. The mixture was extracted with chloroform and the extract dried and evaporated to a pale yellow glass which crystallized from ethanol to yield 60 mg of colorless, stout needles. After recrystallization from ethanol the compound had mp 260–263°. It showed no color with ferric chloride and gave a single spot on tlc.

Anal. Calcd for $C_{28}H_{34}O_{12}$: C, 59.77; H, 6.09; mol wt, 562. Found: C, 59.18; H, 6.32.

Anal. Calcd for $C_{28}H_{34}O_{12}\cdot C_2H_5OH$: C, 59.21; H, 6.62. Found: C, 59.13; H, 6.47.

The infrared spectrum showed a strong peak between 1740 and 1770 cm^{-1}, a shoulder at 1725 cm^{-1}, a strong peak at 1695 cm^{-1}, a band of medium intensity at 1650 cm^{-1}, and a strong peak at 1225 cm^{-1} (acetate C–O stretch).

The mass spectrum of the monoacetate showed a weak molecular ion peak at m/e 562, a weak peak at m/e 544 ($M - 18$), a strong peak at m/e 520 ($M - CH_2CO$), and a small peak at m/e 502 ($M - CH_3COOH$). The acetyl group showed the expected three-proton singlet (δ 2.25) in the nmr spectrum.

Brusatol Triacetate. A.—The mother liquors from the recrystallization of brusatol monoacetate were evaporated to dryness and the residue was treated with a mixture of acetic anhydride, acetic acid, and a few drops of 70% perchloric acid. After 2.5 hr at room temperature the product was isolated in the usual way. The triacetate formed colorless needles from methanol, mp 247–250°. It gave no color with ferric chloride and showed a single spot on tlc.

Anal. Calcd for $C_{32}H_{38}O_{14}$: C, 59.43; H, 5.92. Found: C, 59.12; H, 6.29.

The nmr spectrum showed the presence of the three three-proton singlets of the three acetyl groups (δ 2.03, 2.12, 2.24).

The infrared spectrum showed intense absorption between 1740 and 1770 cm^{-1}, a shoulder at 1725 cm^{-1}, a strong peak at 1695 cm^{-1}, a peak of medium intensity at 1650 cm^{-1}, and a strong absorption at 1225 cm^{-1}.

B.—Acetylation of brusatol by prolonged treatment with acetic anhydride–pyridine, following the course of the reaction with tlc, gave a product which gave an infrared spectrum and behaved on tlc in a way that was identical in every respect with that of the triacetate prepared as in A and gave an nmr spectrum that was identical with that of the mp 247–250° compound; yet it had mp 227–232°. It is apparent that the triacetate can exist in dimorphic forms.

Dihydrobrusatol (Bruceine A).[11]—A solution of 100 mg of brusatol in 15 ml of ethanol was shaken with hydrogen at room temperature and atmospheric pressure in the presence of 100 mg of 10% palladium on charcoal. The reaction was interrupted after the initial rapid absorption had slowed. The filtered solution was evaporated and the residue recrystallized from acetone–methanol. The product (70 mg) formed colorless crystals, mp 270–271°, undepressed on admixture with bruceine A,[12] but lowered when mixed with brusatol: $[\alpha]^{29}D$ −82.5° (*c* 0.8, pyridine) (lit.[11] $[\alpha]^{29}D$ −86.3°).

Anal. Calcd for $C_{26}H_{34}O_{11}$: C, 59.76; H, 6.56; mol wt, 522. Found: C, 59.91; H, 6.55.

Dihydrobrusatol (bruceine A) had λ_{max} 281 mμ (log ϵ 3.96). Its mass spectrum showed a weak molecular ion peak at m/e 522, weak peaks at 504 ($M - 18$), 479 ($M - C_3H_7$), and, at lower m/e values, prominent peaks at 438 ($M - 84$), 151, 85, and the base peak at 57. Its infrared spectrum, which was identical with that of the specimen of bruceine A, showed strong bands at 1757 cm^{-1} (saturated ester) and 1738 cm^{-1} (saturated ester and lactone), a medium band at 1670 cm^{-1} (diosphenol carbonyl) and a weak carbon–carbon double bond peak at 1645 cm^{-1}. The two 3-H singlets at δ 1.90 and 2.11 in brusatol were absent; in their place was a new 6-H doublet (J = 6 cps) at δ 0.94.

Dihydrobrusatol Triacetate.—Acetylation of dihydrobrusatol with pyridine–acetic anhydride at room temperature for 1 hr yielded a substance which could not be crystallized but whose nmr spectrum was clearly that of the monoacetate. When this material was reacetylated with acetic anhydride–perchloric acid, the product, isolated in the usual way, crystallized from methanol as colorless needles, mp 219–221°. The compound gave no color with ferric chloride and showed but one spot on tlc: $[\alpha]^{29}D$ 34.4° (*c* 0.71, chloroform).

Anal. Calcd for $C_{32}H_{40}I_{14}$: C, 59.25; H, 6.22; mol wt, 648. Found: C, 58.93; H, 6.38.

Its mass spectrum showed a weak molecular ion peak at m/e 648 and strong peaks at 588 ($M - CH_3COOH$), 606 ($M - CH_2CO$), and a base peak at m/e 57. The three acetyl groups gave nmr signals (3 H singlets) at δ 2.03, 2.11, 2.25. Dihydrobrusatol triacetate (bruceine A triacetate) appears to exist in dimorphic forms, for Polonsky, *et al.*,[11] report mp 195–200°.

Hydrolysis of Brusatol. Senecioic Acid.—A solution of 165 mg of brusatol in 3 ml of 1 *N* sodium hydroxide was left at room temperature for 24 hr. Acidification of the solution gave a colorless precipitate (61 mg) which was collected. The aqueous layer was extracted continuously with ether to yield a colorless residue. Neither of the materials isolated could be crystallized and an examination of their nmr spectra (in pyridine) showed that the three vinyl methyl groups present in brusatol were still present in these products, but the ester methoxyl group had been lost.

The above residues were combined and saponified by heating on the water bath in 20% aqueous sodium hydroxide for 2 hr. The cooled solution was acidified with hydrochloric acid and the solution steam-distilled. The distillate was saturated with sodium chloride and extracted with ether. Evaporation of the ether left a crystalline residue (5 mg) of senecioic acid, mp 66–68°, undepressed on admixture with an authentic specimen and having an infrared spectrum identical with that of senecioic acid.

Examination of the Water-Soluble Fraction.—The dark brown, water-soluble syrup remaining after methylene chloride extraction of brusatol was intensely bitter. This material gives a purple-brown color with sulfuric acid, an observation that indicates the presence of material that belongs to the general group of compounds known to occur in simaroubaceous plants that give this characteristic color reaction.[3,13] It is to be noted that in early studies on the constituents of *B. sumatrana*[14] there was described a substance, "yatanoside," regarded at that time as a glycoside, that showed a violet color with concentrated sulfuric acid.

Further study of the extracts of *B. sumatrana* is in progress.

Registry No.—**3**, 14907-98-3; **3**-monoacetate, 15156-68-0; **3**-triacetate, 14907-99-4; dihydrobrusatol, 14908-00-0; dihydrobrusatol triacetate, 14908-01-1.

Acknowledgments.—K. Y. S. thanks the Committee on International Exchange of Persons for the award of a Fulbright Research Scholarship. This study was supported by a grant from the National Institutes of Health, Institute for Allergy and Infectious Diseases, AI-07435. Analyses were performed by Miss Heather King, U. C. L. A.

(12) We thank Mme. Polonsky for the gift of a specimen of bruceine A from *B. amarissima*.

(13) T. A. Geissman and G. A. Ellestad, *Tetrahedron Letters*, 1038 (1962).

(14) C. K. Liang, *J. Chinese Chem. Soc.*, **16**, 53 (1949); *Chem. Abstr.*, **43**, 9377 (1949).