Anal. Calcd. for $C_{13}H_{12}O_6$: C, 59.1; H, 4.6. Found: C, 58.8; H, 4.7.

Scopoletin-4-carboxylic acid (IV). Crude ester III (3.77 g., 14.3 moles) was slurried in 50 ml. of water and 2.5*M* sodium hydroxide solution (11.5 ml.) was added. After 24 hr. at room temperature, the deep red solution was acidified, the precipitate was removed by filtration, washed with cold water, and air-dried to give 2.50 g. (74% crude yield) of red powder, m.p. 292–293° dec. Trituration with a small amount of cold ethanol provided a yellow-orange powder, m.p. 297–299° dec., while recrystallization from ethanol gave bright yellow needles, m.p. 300–301° dec.

Anal. Calcd. for $C_{11}H_8O_6$: C, 55.9; H, 3.4. Found: C, 55.9; H, 3.5.

Scopoletin (V). The crude acid IV (300 mg.) was mixed with 12 g. of quinoline and 500 mg. of copper powder and boiled under reflux until carbon dioxide evolution ceased (about 30 min.). The cooled mixture was diluted with 100 ml. of chloroform, filtered, and extracted with 10% sulfuric acid. After washing further with water, the chloroform solution was dried, decolorized, and the solvent evaporated under nitrogen. The solid residue was triturated with a few drops of ethanol and filtered. The yield of crystalline scopoletin, m.p. 201–202°, was 200 mg. (82%). The melting point of a mixture with authentic scopoletin (m.p. 203–204°) was undepressed, and infrared spectra and chromatographic properties of the two were identical.

Another portion of IV (300 mg.) was thoroughly mixed with 500 mg. of copper powder and warmed over a small flame. As decomposition proceeded, a yellow solid sublimed onto the walls of the tube. When gas evolution had ceased, the tube was cooled and the contents extracted repeatedly with hot ethanol until extracts no longer were appreciably fluorescent. The combined extracts were filtered, ethanol removed under nitrogen, and the residue recrystallized from glacial acetic acid. The yield of scopoletin, m.p. 198–200°, was 150 mg. (60%).

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Bitter Principles of the Simaroubaceae. I. Chaparrin from *Castela Nicholsoni*

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A bitter lactone, chaparrin, has been isolated from *Castela Nicholsoni*, and converted into a bis-dehydration product, chaparrol, also a lactone. The uncharacterized crystalline material accompanying chaparrin yielded glaucanol on treatment with acid, suggesting the presence in *Castela Nicholsoni* of glaucarubol.

Castela Nicholsoni Hook ("Chaparro amargosa") is a bitter shrub of the family Simaroubaceae, native of Mexico. Like a number of species of other genera of this family (*Simarouba, Brucea, Ailanthus, Quassia*), *Castela Nicholsoni* has found extensive use in folk medicine:[2] most of these drugs are used as amoebicides in the treatment of dysentery,[3–12] and several of them have yielded crystalline principles that have been found to be highly effective amoebicidal agents.

C. Nicholsoni has been examined chemically and pharmacologically by numerous investigators. Bosman[13] reported the isolation of three crystalline compounds: castelin, a glycoside; castelagenin, its aglycon; and castelamarin. To the latter, a bitter lactone giving a blue color with concentrated sulfuric acid (characteristic of many of the compounds of this group), was assigned the unlikely structure of the lactone of 2-hydroxy-3-methoxycyclohexaneacetic acid. The synthesis of a compound with this structure was reported by Paranjape *et al.*,[14] and found not to be identical with the natural lactone.

In 1944, Alles and Saunders[15] re-examined "chaparro amargosa" and succeeded in isolating two substances. One of these, m.p. 286–288°, gave a blue coloration with concentrated sulfuric acid; the other, m.p. 280–287°, gave no color with sulfuric acid. Their study was terminated before adequate purification of these materials could be carried out.

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Studies in this laboratory have now led to certain definite results. A bitter lactone, called chaparrin, has been isolated as a pure compound, and its composition and functional groups have been established. Among other, incompletely characterized fractions of the crystalline materials isolated from the plant, the presence of glaucarubol has been inferred by the formation of glaucanol, identified as described below, from a poorly characterized fraction. Glaucarubin, from which glaucarubol is derived, is a constituent of *Simarouba glauca*.[16,17] Like chaparrin, it is a bitter lactone that gives a deep blue coloration with concentrated sulfuric acid.

Chaparrin is a colorless crystalline compound with m.p. 309–310°. It is dextrorotatory (in pyridine), and dissolves with surprising ease in dilute aqueous sodium hydroxide. On acidification chaparrin is regenerated. The presence of a δ-lactone is indicated by a strong, symmetrical infrared absorption band at about 1720 cm.$^{-1}$ Other features of the complex infrared absorption spectra afford little useful structural information: strong hydroxyl group absorption in the 3400 cm.$^{-1}$ region, indications of absorption due to carbon-linked methyl groups at about 1400 cm.$^{-1}$ and carbon-oxygen stretching in the 1250 cm.$^{-1}$ region (most of the infrared spectra were determined in potassium bromide disks because of low solubility of many of the compounds in chloroform) confirm the presence of structural features disclosed by chemical studies. The infrared absorption of the sodium salt of the acid derived by opening of the lactone ring showed the characteristic absorption for hydroxyl groups and the carboxylate ion. A carbon-carbon double bond is suggested by a weak but sharp peak at 1600 cm.$^{-1}$, but no carbonyl absorption of other kinds was observed.

Chaparrin was difficult to purify and reproducible analytical values were not easy to obtain. An indication of a possible reason for this was found in the observation that at least one sample of chaparrin, of proper melting point, showed a small absorption peak in the infrared that could be ascribed to the carboxyl group. This suggests that incomplete lactonization (which would probably not affect the melting point of about 300° or higher) may introduce small and variable amounts of the elements of water. It was always observed that very careful drying of analytical samples was necessary. The results of numerous concordant carbon-hydrogen analyses led to the conclusion that chaparrin has the composition $C_{20}H_{28}O_7$.

Several comments on this formula are pertinent: quassin, the bitter lactone of *Quassia amara*,[18,19] is a dimethyl ether of a (hypothetical) substance of the composition $C_{20}H_{24}O_6$; glaucarubol, of which glaucarubin is the α-methyl-α-hydroxybutyric ester,[16] has the composition $C_{20}H_{28}O_8$; and simarolide, a bitter lactone recently isolated from *Simarouba amara*,[20] is the monacetate of a substance of the composition $C_{20}H_{30}O_6$. It is apparent that there are present in various plants of the Simaroubaceae a group of compounds that appear to be closely allied in structure: They possess or are derived from a C_{20} structure, are lactones, and many of them show a striking red or blue coloration with concentrated sulfuric acid. Moreover, numerous studies on *Brucea* spp.[7–12] have resulted in the isolation of a number of crystalline compounds, many of uncertain homogeneity, most of which show the same kind of color reaction and possess compositions approximating those given above.

Chaparrin is readily acetylated to yield two isomeric pentaacetyl derivatives, m.p. 226–228° and m.p. 135–137°. Both of these show the complete absence of hydroxyl groups by their infrared spectra, and both of them yield chaparrin upon alkaline hydrolysis. Their composition shows that chaparrin contains five hydroxyl groups and thus the seven oxygen atoms are completely accounted for. Benzoylation of chaparrin yields the expected pentabenzoate.

When chaparrin is treated with hot, dilute aqueous hydrochloric acid, two crystalline compounds are formed. One of these, formed in largest amount and called chaparrol, is a bitter lactone, readily soluble in aqueous alkali but insoluble in sodium bicarbonate solution; it gives no coloration with concentrated sulfuric acid, and its infrared absorption spectrum shows the presence of hydroxyl groups and the lactone ring. Chaparrol has the composition $C_{20}H_{24}O_5$, and forms a triacetate which shows no hydroxyl absorption in the infrared. The formation of chaparrol from chaparrin clearly involves the loss of two molecules of water. The ultraviolet absorption spectrum of chaparrol shows two maxima at 271 and 278 mμ (log ε 2.54 and 2.50). The spectrum is strikingly similar to that of 6-methyl-1,2,3,4-tetrahydronaphthalene and is evidence for the presence of an aromatic ring, perhaps in a tetralin-like structure, in chaparrol.

It is of interest to note that glaucanol, formed from glaucarubol by treatment of the latter with aqueous acid, has the composition $C_{20}H_{24}O_6$, and thus differs from glaucarubol by the elements of two molecules of water.

The product formed in small amount along with chaparrol has not been satisfactorily characterized, but its elementary composition appears to be $C_{20}H_{26}O_6$. It is thus a mono-dehydration product of

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chaparrin, but is unusual in that its absorption in the infrared is that of an α,β-unsaturated δ-lactone. Further comment on this compound must be reserved until it can be obtained in sufficient amount for closer study.

Among the lower-melting fractions of crystalline material that accumulate in the purification of chaparrin was a material that could be further recrystallized to a melting point of 290–291°. A mixed melting point with authentic glaucarubol (m.p. 292–293°) showed no depression, but its elementary analysis was intermediate between those of glaucarubol and chaparrin. That this material is impure glaucarubol is probable, since it could be converted by acid hydrolysis into glaucanol, identified by the identity of its infrared spectrum with that of an authentic sample, a mixed melting point, analysis, and the preparation of glaucanol acetate (identical with authentic material by mixed melting point and infrared spectra).

Besides chaparrin (and perhaps glaucarubol), at least one other constituent is present in *C. Nicholsoni*. It was isolated in small yield from plant extracts from which the bulk of the readily crystallizable material had been removed, and had m.p. 263–265°. It was a bitter substance, soluble in alkali but not in aqueous sodium bicarbonate. It was clearly distinguished from chaparrin and glaucarubol by the fact that it gave no coloration with concentrated sulfuric acid. Its elementary analysis gave figures in good agreement with the formula $C_{22}H_{30}O_7$ (a monoacetate of $C_{20}H_{28}O_6$).

Since chaparrin is best regarded as a pentahydroxy lactone, the formation of two isomeric pentaacetates is remarkable. The most reasonable interpretation of this result at the present time is that the lactone ring of chaparrin is sufficiently labile to permit the formation of pentaacetates in which the lactonic oxygen atom of one is found in an acetyloxy group of the other. The regeneration of chaparrin from both acetates shows that no deep-seated change occurs in the acetylation.

EXPERIMENTAL

Isolation of chaparrin: The milled whole aerial part of *Castela Nicholsoni* was extracted with methanol in 9-kg. batches in a stainless steel extractor (percolator) for 48 hr. The concentrated extract was treated with water, reconcentrated, and made up to 3 l. with water and allowed to stand overnight. After separation of a tarry deposit the filtrate was treated with about 25 ml. of 1*M* lead acetate solution, filtered through Celite, and treated again with lead acetate until precipitation was completed. Excess lead was removed with sulfuric acid (to a *p*H of 3.5 to 4.0) and the filtrate was concentrated to a volume of about 300 ml. The concentrated solution was shaken with about one-quarter its volume of chloroform, when a white semicrystalline solid separated. After centrifugation, the solid was shaken with a 1:5 mixture of chloroform and water and then with acetone. The final crude product was a solid with a m.p. of about 200–265°, and amounted to about 6.5 g.

The crude product was dissolved in hot methanol and the filtered solution concentrated by distillation to about 2 l. After 24 hr. a crop of crystalline material was collected, washed with methanol, and dried. Further concentration of the filtrates yielded successive crops of crystalline materials.

Those fractions having melting points of 290° or above were combined and recrystallized several times from methanol, to yield chaparrin as tiny white needles, m.p. 309–10°, $[\alpha]_D^{25.5}$ 45.2° (*c*, 0.2 in pyridine).

Anal. Calcd. for $C_{20}H_{28}O_7$: C, 63.14; H, 7.42; mol. wt. 380. Found: C, 62.95; 63.18, 62.98, 62.97; 63.26, 63.13. H, 7.49, 7.32, 7.53, 7.63, 7.02.

The equivalent weight was determined by treatment with 0.0494*N* sodium hydroxide for 6 hr. at 60°, and back titrating with standard acid: 383. C-methyl number, found: 1.73, 1.71, 1.80.

Acetylation of chaparrin. (A) *With acetic anhydride–pyridine.* A solution of 500 mg. of chaparrin in 2 ml. of pyridine and 4 ml. of acetic anhydride was heated on the steam bath for 90 min. Removal of the solvents left a gummy residue which was taken up in 3 ml. of chloroform and passed through a column of 15 g. of acid-washed alumina with 100 ml. of chloroform. The 650 mg. of white solid recovered from the eluate was recrystallized from petroleum ether (b.p. 30–60°) to yield 350 mg. of white needles, m.p. 226–8°, $[\alpha]_D^{25.6}$ 40.0 (*c*, 0.9 in pyridine). Its infrared absorption (in chloroform) showed the complete absence of hydroxyl groups.

Anal. Calcd. for $C_{20}H_{23}O_7(COCH_3)_5$: C, 61.02; H, 6.44, OAc, 36.43. Found: C, 60.86, 61.48, 61.29, 61.70; H, 6.64, 6.89, 6.65, 6.62; OAc, 36.27; C-Methyl, 6.25, 6.24.

After the separation of the 226–228° acetate, the mother liquors were concentrated to yield a second crop, m.p. (after recrystallization from petroleum ether (b.p. 30–60°) 135–137°, $[\alpha]_D^{25.5}$ 41.2 (*c*, 0.75 in pyridine). The infrared absorption spectrum of this acetate was very similar to, but not completely identical with that of the 226–228° acetate; no hydroxyl absorption was present.

Anal. Calcd. for $C_{20}H_{23}O_7(COCH_3)_5$: C, 61.02; H, 6.44; OAc, 36.43. Found: C, 60.66, 60.12; H, 6.60, 6.50; OAc, 35.83, 35.91; C-Methyl, 6.44.

(B) *With acetic anhydride–sodium acetate.* A solution of 500 mg. of chaparrin in 4 ml. of acetic anhydride and 25 mg. of sodium acetate was refluxed for 90 min. The reaction mixture was decomposed with water to yield 300 mg. of white solid, and saturation of the filtrate with sodium chloride gave an additional 225 mg. The crude material was worked up as described above (A) to yield 70 mg. of acetate, m.p. 226–228° and 400 mg. of acetate, m.p. 135–137°.

Hydrolysis of chaparrin acetates. (A) A solution of 60 mg. of the acetate m.p. 226–228° in 3 ml. of 1*N* alcoholic potassium hydroxide was heated for an hour. The ethanol was removed and the residue dissolved in 5 ml. of water. Acidification gave 25 mg. of crystalline material, m.p. 303–304°, undepressed in a mixture (m.p. 304–305°) with pure chaparrin. The infrared absorption spectra of the product and of pure chaparrin were identical in every detail.

(B) Alkaline hydrolysis of 135–137° acetate was carried out as described in (A). The product melted at 299–300° and a mixture with chaparrin also melted at 299–300°. The infrared absorption curves of the product of this hydrolysis and of chaparrin were nearly identical, with the exception that the regenerated material showed a small shoulder at about 1700 cm.$^{-1}$ absent from most chaparrin samples (but observed in one or two).

Anal. Calcd. for $C_{20}H_{28}O_7$: C, 63.14; H, 7.42. Found: C,63.06; H, 7.65.

In another experiment, the regenerated chaparrin (m.p. 295–296°) gave the following analytical figures: C, 61.84; H, 7.17.

Chaparrin benzoate. Benzoylation of chaparrin (200 mg.) with benzoyl chloride–pyridine in the usual way yielded 260 mg. of crude product which was crystallized three times from ethanol to give 110 mg. of white crystals, m.p. 159–160°. Its infrared spectrum showed no hydroxyl group absorption.

Anal. Calcd. for $C_{20}H_{23}O_7(COC_6H_5)$: C, 71.90; H, 5.45. Found: C, 71.28; H, 5.60.

Acid treatment of chaparrin: Chaparrol. A slurry of 500 mg. of chaparrin in 50 ml. of 0.1*N* hydrochloric acid was heated to boiling; in about 20 min. a clear solution was formed. After an additional 1.5 hr. of refluxing the solution was cooled and extracted with chloroform. The dried chloroform solution was chromatographed on 15 g. of acid-washed alumina and eluted with 150 ml. of chloroform. Removal of the solvent left 385 mg. of yellowish solid. This was dissolved in 3 ml. of ethyl acetate and cooled, when 250 mg. of crystalline material was obtained. Recrystallization from petroleum ether (b.p. 60–80°) afforded chaparrol, colorless crystals, m.p. 221–222°, $[\alpha]_D^{25.5}$ 119.0° (*c*, 0.94 in pyridine).

Anal. Calcd. for $C_{20}H_{24}O_5$: C, 69.92; H, 6.99; mol. wt., 344. Found: C, 69.74, 69.95; H, 7.18; mol. wt. (Rast), 341, 337; C-Methyl, 1.51, 1.52.

The ethyl acetate filtrates were evaporated to dryness and the residue crystallized twice from toluene to yield a *product*, m.p. 159–60°.

Anal. Found: C, 66.40; H, 6.68.

Chaparrol acetate. Acetylation of 200 mg. of chaparrol with acetic anhydride–pyridine, followed by chromatography of the product over alumina (chloroform) yielded 240 mg. of product which after two recrystallizations from petroleum ether (b.p. 30–60°) gave 150 mg. of white needles, m.p. 170–171°, $[\alpha]_D^{25.5}$ 167.0° (*c*, 0.94 in pyridine).

Anal. Calcd. for $C_{20}H_{21}O_5(COCH_3)_3$: C, 66.39; H, 6.38; OAc, 27.45. Found: C, 66.46, 65.85; H, 6.56, 6.52; OAc, 27.35; C-Methyl, 4.78.

Formation of glaucanol from lower-melting fractions. A crystalline fraction, m.p. 280–283°, from the purification of chaparrin, was recrystallized from methanol to give white platelets, m.p. 290–291°. Its mixed melting point with a sample of glaucarubol (m.p. 292–293°) showed no depression.

Anal. Calcd. for $C_{20}H_{28}O_8$, C, 60.60; 7.12. Calcd. for $C_{20}H_{28}O_7$, C, 63.14; 7.42. Found: (m.p. 290–291°) C, 61.93; 61.87; H, 7.23, 7.92.

Acid hydrolysis of material m.p. 280–283°. Hydrolysis of 200 mg. of a crystalline fraction, m.p. 280–283°, from the purification of chaparrin, by the use of 0.1*N* hydrochloric acid as described for chaparrol, yielded 150 mg. of crystalline material after chromatography on alumina. This melted at 230–233° before further purification but after two recrystallizations from ethanol it formed white platelets with m.p. 259–60°. A mixed m.p. of a sample of glaucanol,[21] m.p. 229–233°, and the m.p. 230–233° material was unchanged.

The m.p. 259–260° material also showed no depression in melting point on mixing with a specimen of glaucanol,[22] m.p. 252–253°. Comparison of the infrared spectra of our sample and the authentic specimen showed their complete identity.

Anal. Calcd. for $C_{20}H_{24}O_6$: C, 66.60; H, 6.66. Found (m.p. 259–260°): C, 66.49; H, 6.59.

Glaucanol acetate. Acetylation of the sample of glaucanol m.p. 259–60°, with acetic anhydride–pyridine yielded (after purification over alumina) 35 mg. of crystalline solid. After two recrystallizations from petroleum ether (b.p. 30–60°) this yielded 20 mg. of white crystals, m.p. 209–210°. A mixed melting point with authentic glaucanol acetate[22] (m.p. 210–211°) showed no depression, and the infrared spectra of the two specimens were identical in every detail.

Anal. Calcd. for $C_{20}H_{20}O_6(COOCH_3)_4$: C, 63.63; H, 6.06. Found: C, 63.58; H, 5.66.

Compound m.p. 263–265°. After recovery of the crude chaparrin that separated upon the addition of chloroform to the first concentrated extract, the aqueous solution was extracted continuously with chloroform for 72 hr. The dried chloroform extract was evaporated and the nearly solid residue triturated with a little ethanol. A white crystalline material separated, and was collected and recrystallized twice from ethanol, when it formed white platelets, m.p. 263–265°. This material was markedly different from chaparrin (both the pure material and the lower-melting fractions), which gave an intense blue-violet color with concentrated sulfuric acid. This new compound gave no color under the same conditions. It was bitter and had the solubility behavior expected of a lactone (soluble in dilute alkali but not in aqueous bicarbonate). Its infrared absorption was markedly different from that of chaparrin and indeed was not that to be expected of a simple δ-lactone; two peaks were present in the carbonyl region, one at 1695 cm.$^{-1}$, the other at about 1715 cm.$^{-1}$ The presence of prominent peaks in the curve at 1236, 1245, and 1265 cm.$^{-1}$ is suggestive of an acetyloxy grouping, and the analytical figures are in good agreement for a monoacetate of a C_{20}-compound.

Anal. Calcd. for $C_{22}H_{30}O_7$: C, 65.00; H, 7.45. Found: C, 65.06; H, 7.38.

Since only 30 mg. of this compound has been in hand, further study must await the isolation of more material.

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(21) This specimen was kindly provided by Dr. E. A. Ham, Merck Sharpe and Dohme Research Laboratories.

(22) This was provided by Prof. P. Yates, who has recently undertaken further study of *Simarouba glauca*. This difference in melting point of the two "authentic" samples is consistent with our experience that the purification of crude glaucanol raises its melting point some 30°.