Occurrence of Tomentosic Acid in Extracts of *Bixa orellana*

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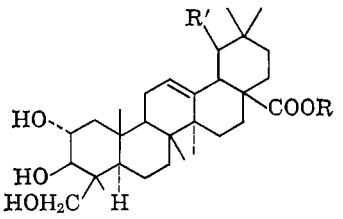
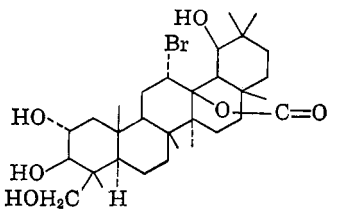
In the course of a program designed to discover pharmacologically active natural products, we fractionated the crude residue resulting from solvent extraction of the ground root of the Central American plant *Bixa orellana*. After repeated countercurrent distributions in a water–acetone–Skellysolve B system, a crystalline product was isolated, whose analytical values and spectral properties could be interpreted as that of a polyhydroxy acid, $C_{30}H_{48}O_6$.

Acetylation with acetic anhydride and pyridine gave an amorphous product which was determined to be a triacetate by analysis and by its n.m.r. spectrum.[1] Also evident in the n.m.r. spectrum was absorption by one olefinic hydrogen atom split into a triplet by two adjacent hydrogens (centered at 307 c.p.s.), and six methyl groups (46, 54, 54, 56, 66, and 68 c.p.s.). The hydroxy acid formed a crystalline methyl ester on long standing with excess diazomethane in methanol–ether, but was recovered unchanged after 0.5 hr. in ethereal diazomethane, indicating the hindered nature of the carboxyl group. A crystalline bromo lactone was formed on treatment with bromine in acetic acid.

While the above work was in progress, papers by Row and Rao[2] appeared describing the isolation and structure proposal (Ia) for a new triterpene acid, $C_{30}H_{48}O_6$, obtained from *Terminalia tomentosa* Wight et Arn. After noting the similarity of our product from *B. orellana* with their tomentosic acid, we were able to compare directly samples[3] of tomentosic acid, its methyl ester (Ib), and bromo lactone (II) with the corresponding products from *B. orellana* and find they are identical by the following criteria: infrared spectral comparisons, mixture melting points, thin layer chromatographic behavior, and color reactions with sulfuric acid. In addition, we found small amounts of another triterpene acid, $C_{30}H_{48}O_5$, in the *B. orellana* product, which may correspond to arjunolic acid (Ic),[4] also found in *Terminalia*.[2] We were unable, however, to obtain enough of it for positive identification.



Ia, R = H; R′ = OH
b, R = CH_3; R′ = OH
c, R = R′ = H



II

The *Bixaceae* represent a small family of the order *Parietales* of essentially New World origin, while the *Combretaceae* (*Terminalia* spp.) belong to the Old World order, *Myrtales*.[5] It seems quite an unexpected coincidence that the same new triterpene acid should occur in both, but provides an example of the similarity of biogenetic pathways in plants growing on opposite sides of the earth.

Experimental[6]

Isolation of Tomentosic Acid from *Bixa orellana*.—A total of 1850 g. of dried, milled whole root of *Bixa orellana* was extracted by stirring 5 hr. under reflux three times with 13 l. of 95% ethanol. The combined ethanol filtrates (34 l.) were evaporated *in vacuo* to about 1-l. volume which was then diluted with 2 l. of water and extracted three times with 1.75 l. of diethyl ether. The combined ethereal extracts were evaporated *in vacuo* to a brown tar which was dissolved in 750 ml. of 80% aqueous *t*-butyl alcohol and lyophilized to obtain 32.5 g. of yellow powder. A total of 445 g. of dried, milled root bark was extracted in proportionately the same way to obtain 12.5 g. of yellow powder. The combined yellow powders (45 g.) were fractionated by a three-tube double-withdrawal countercurrent extraction scheme using 750 ml. of each phase formed by mixing water, acetone, and Skellysolve B in the ratio of 2:5:3 by volume. The lower phase fractions on evaporation gave 17.4 g. of an amber gum. This material was then subjected to 200 transfers in a 200-tube automatic countercurrent distribution apparatus using the same solvent system as above. The contents of tubes 41–80 gave on evaporation 3.64 g. of yellow powder. The contents of tubes 20–40 gave on evaporation 2.83 g. which was again subjected to 200 transfers in the same manner to provide an additional 300 mg. of material in tubes 41–80. The combined 41–80 fraction, 3.94 g., was again subjected to 200 transfers in the same solvent system as above, and the contents of tubes 20–60 were recycled to a total of 1000 transfers. The major band, contained within tubes 110–200, was pooled and saturated with sodium chloride, and the organic phase was separated, filtered, and concentrated to a small volume. After refrigeration, a total of 1.06 g. of crystals was collected by filtration, m.p. 295–300°. A 493-mg. sample was recrystallized from 60% aqueous ethanol to give 406 mg. of colorless needles, m.p. 298–303°. A further recrystallization from acetone gave a different polymorphic modification, colorless rods, m.p. 315–330°. This material was identical with an authentic sample of tomentosic acid[3] in its infrared absorption spectrum (Nujol mull), and thin layer chromatographic behavior (R_f 0.23 on silica gel developed with 5% methanol in ethyl acetate). The melting point was not depressed on admixture with tomentosic acid.

Anal. Calcd. for $C_{30}H_{48}O_6$: C, 71.39; H, 9.59. Found: C, 71.65; H, 10.24.

Methyl Tomentosate.—A 30-mg. sample of the crystalline *Bixa* compound above, in 1 ml. of methanol, was treated with excess ethereal diazomethane, essentially as in ref. 2, and the crude product was chromatographed on 5 g. of Florisil. Elution with 10% acetone in Skellysolve B gave a small crystalline peak, 6 mg., m.p. 222–224°, after recrystallization from methanol, followed by 25 mg. of crystalline methyl tomentosate, eluted with 30%

(1) Measured in deuteriochloroform solution on a Varian A-60 spectrophotometer. Three acetate methyl groups were evident at 119, 121.5, and 126 c.p.s. downfield from tetramethylsilane.

(2) L. R. Row and G. S. R. Rao, *Tetrahedron Letters*, **No. 27**, 12 (1960); *Tetrahedron*, **18**, 827 (1962).

(3) Kindly furnished by Dr. L. R. Row.

(4) F. E. King, T. J. King, and J. M. Ross, *J. Chem. Soc.*, 3995 (1954).

(5) We are indebted to Professor W. B. Drew, Chairman of the Department of Botany and Plant Pathology, Michigan State University, East Lansing, Mich., for this information.

(6) Melting points are corrected and observed on a Kofler hot-stage. Florisil is a synthetic magnesia–silica gel manufactured by the Floridin Co., Pittsburgh, Pa. Skellysolve B is a saturated hydrocarbon fraction, b.p. 60–71°, from Skelly Oil Co., Kansas City, Mo.

acetone-Skellysolve B, m.p. 217–220° after recrystallization from acetone (lit.[2] m.p. 221–222°). The infrared absorption spectrum (Nujol mull) was identical with that of an authentic sample,[3] as was the mobility (R_f 0.41) on silica gel developed as above.

Tomentosic Bromo Lactone.—A 77-mg. sample of the *Bixa* acid was brominated in acetic acid containing sodium acetate essentially as in ref. 3. The crude product, 88 mg., was chromatographed on Florisil and the major peak, eluted with 15% acetone in methylene chloride, was recrystallized twice from methanol, m.p. 233–235°, $[\alpha]_D$ +49° ($CHCl_3$). These data, as well as the infrared absorption spectra and behavior on silica gel thin layer plates, are substantially identical with those of an authentic sample of tomentosic bromo lactone.[3]

Anal. Calcd. for $C_{30}H_{47}BrO_5$: C, 64.14; H, 8.73. Found: C, 63.48; H, 8.35.

Tomentosic Acid Triacetate.—A 50-mg. sample of the *Bixa* acid was treated overnight at 25° with 1 ml. each of acetic anhydride and pyridine. Slow dilution with water gave an amorphous solid which was filtered, washed with water, and dried to yield 57 mg., m.p. about 150°. It could not be satisfactorily recrystallized. The n.m.r. spectrum in deuteriochloroform solution is summarized in the discussion section. Optical rotatory dispersion data indicated a positive plain curve: $[\alpha]_{589}$ +100°, $[\alpha]_{350}$ +175°, $[\alpha]_{310}$ +1565°.

Anal. Calcd. for $C_{36}H_{54}O_9$: C, 68.54; H, 8.63. Found: C, 69.00; H, 8.83.

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A Michael Addition with 6-Chloropurine[1]

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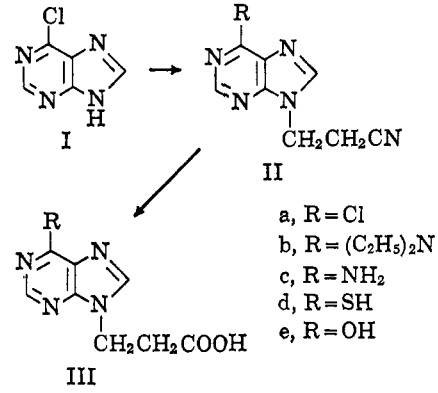
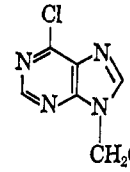
Since 6-chloropurine (I) is readily alkylated primarily at the 9-position,[1a,2,3] it is a versatile precursor for the synthesis of 6-substituted purines bearing a 9-alkyl group; this class of compounds has been of interest for a variety of biological studies.[3] Furthermore, 6-chloropurine will add to dihydropyran with acid catalysis in the usual orientation of addition to this enol ether.[4] For some biological studies related to those previously described,[3a,b] some 6-substituted 9H-purin-9-ylpropionic acids (III) were needed. If 6-chloropurine (I) could be added to acrylonitrile by base-catalyzed Michael addition, the product IIa would be a suitable precursor for the desired purin-9-ylpropionic acids (III).



Initial study on the addition of 6-chloropurine (I) to acrylonitrile in N,N-dimethylformamide catalyzed by potassium carbonate failed to give the desired adduct IIa; it was noted that an insoluble potassium salt formed which was apparently too insoluble to react. In contrast, the potassium salt was relatively soluble in dimethyl sulfoxide and initial conditions for addition gave a 14% yield of 6-chloro-9H-purin-9-ylpropionitrile (IIa, Table I, run 1); a study of the reaction variables finally led to optimum conditions which gave a 73% yield of IIa when a catalytic amount (6 mole %) of potassium carbonate was employed.

TABLE I

REACTION CONDITIONS *vs.* YIELDS FOR PREPARATION OF IIA[a]



Run	Molar ratio of I–acrylonitrile–K_2CO_3	Reaction time, hr.	% yield
1	1:1:0.06	40	14
2	1:2:0.06	72	59
3	1:5:0.12	1.5	18[b]
4	1:5:0.12	20	48
5	1:5:0.06	48	63
6	1:5:0.06	74	73
7	1:10:0.06	70	78[c]

[a] All runs were made with 3.3 mmoles of 6-chloropurine (I) in 5 ml. of dimethyl sulfoxide at room temperature, then processed as described for IIa. The yields are recorded for once-recrystallized product melting no more than 10° below the analytical sample, unless otherwise indicated. [b] This product contained some unchanged 6-chloropurine. [c] This product was impure and appeared to contain some polymeric material.

That IIa was a 9-substituted and not a 7-substituted purine was shown by reaction with diethylamine in boiling methanol[3a]; the resultant 6-diethylaminopurine (IIb) showed ultraviolet peaks in agreement with a 9-substituted purine,[5] but not a 7-substituted purine.

By reaction with thiourea in boiling ethanol,[2,3] the 6-chloropurine (IIa) was converted to the corresponding 6-purinethiol (IId) in 76% yield. A 1-hr. reaction of

(1) (a) Paper XXI of the series on Nonclassical Antimetabolites; for paper XX, see B. R. Baker, P. M. Tanna, and G. D. F. Jackson, *J. Pharm. Sci.*, in press. (b) This work was generously supported by Grant No. CA-05867 from the National Cancer Institute, U. S. Public Health Service.

(2) (a) J. A. Montgomery and C. Temple, Jr., *J. Am. Chem. Soc.*, **83**, 630 (1961); (b) H. J. Schaeffer and R. D. Weimar, Jr., *ibid.*, **81**, 197 (1959); (c) G. B. Brown and V. S. Weliky, *J. Biol. Chem.*, **204**, 1019 (1953); (d) B. R. Baker and K. Hewson, *J. Org. Chem.*, **22**, 959 (1957); (e) B. R. Baker, K. Hewson, H. J. Thomas, and J. A. Johnson, Jr., *ibid.*, **22**, 954 (1957).

(3) (a) B. R. Baker and H. S. Sachdev, *J. Pharm. Sci.*, **52**, 933 (1963), paper XIII of this series; (b) B. R. Baker and P. M. Tanna, *ibid.*, in press, paper XIX of this series; (c) H. J. Schaeffer and R. Vince, *J. Med. Chem.*, **8**, 33 (1965); (d) H. J. Schaeffer and P. S. Bhargava, *Biochemistry*, **4**, 71 (1965); H. J. Schaeffer, S. Marathe, and V. Alks, *J. Pharm. Sci.*, **53**, 1368 (1964).

(4) R. K. Robins, E. F. Godefroi, E. C. Taylor, L. L. Lewis, and A. Jackson, *J. Am. Chem. Soc.*, **83**, 2574 (1961).

(5) (a) B. R. Baker, R. E. Schaub, and J. P. Joseph, *J. Org. Chem.*, **19**, 638 (1954); (b) L. B. Townsend, R. K. Robins, R. N. Leoppky, and N. J. Leonard, *J. Am. Chem. Soc.*, **86**, 5320 (1964).