Reprinted from JOURNAL OF NATURAL PRODUCTS, Vol. 43, No. 4, Jul-Aug, 1980

THE ANTINEOPLASTIC QUASSINOIDS OF *SIMABA CUSPIDATA* SPRUCE AND *AILANTHUS GRANDIS* PRAIN

JUDITH POLONSKY[1], ZOIA VARON and CHRISTIAN MORETTI[2]

Institute de Chimie des Substances Naturelles, CNRS 91190 Gif sur Yvette, France

GEORGE R. PETTIT[3], CHERRY L. HERALD and JOHN A. RIDEOUT

Cancer Research Institute and Department of Chemistry, Arizona State University, Tempe, Arizona 85281

SHRI B. SAHA[4] and HARI N. KHASTGIR[5]

University of North Bengal, Raja Rammohunpur, Darjeeling, India

ABSTRACT.—The South American *Simaba cuspidata* Spruce and North Indian *Ailanthus grandis* Prain were investigated as sources of potentially useful antineoplastic agents. Both of these Simaroubaceae plant species were found to produce 6α-tigloyloxychaparrinone (**4a**) and the new quassinoid 6α-tigloyloxychaparrin (**3b**). The latter structure was determined by interpretation of spectral data and oxidation to 6α-tigloyloxychaparrinone (**4a**). While both glycol **3b** and α-ketol **4a** were found to significantly inhibit growth of the murine P388 lymphocytic leukemia cell line, only the α-ketol (**4a**) inhibited growth of the corresponding *in vivo* system.

A significant number of the tropical to subtropical trees and shrubs that generally characterize the relatively small (some 120 species in 20–24 genera) Simaroubaceae family (2) are now known to contain potentially useful medicinal agents ranging from anthelmintic (3) and antiamebic (4) to antineoplastic (5–13) agents. The quassinoid constituents such as bruceantin (1), now undergoing clinical trial by the U. S. National Cancer Institute (8), have been of special interest. In a recent study of the French Guianan *Simarouba amara* Aubl. (Simaroubaceae), we (10) isolated two new quassinoids, namely the cell growth inhibitory [P388 ED_{50} 0.95 μg/ml, (14)] 13,18-dehydroglaucarubinone (**2**) and the essentially inactive 2'-acetylglaucarubin (**3a**). Further efforts directed at uncovering potentially useful cancer chemotherapeutic drugs led us to explore two more hitherto unevaluated Simaroubaceae members.

Stem bark of *Simaba cuspidata* Spruce, collected in French Guiana, was extracted with hexane, followed by hot water. The aqueous solution was concentrated and extracted with chloroform. Evaporation of the chloroform yielded a residue which, when crystallized, yielded the new quassinoid 6α-tigloyloxychaparrin (**3b**). The molecular formula was found by elemental and mass spectral analyses to be $C_{25}H_{34}O_9$. An infrared spectrum showed the presence of carbonyl groups at 1740 (δ-lactone) and 1700 (α,β-unsaturated ester) cm^{-1}. The ultraviolet absorption at 220 nm (log $\epsilon = 3.11$) was attributed to the α,β-unsaturated ester. Presence of a tiglic acid ester was shown by the mass spectral fragmentation

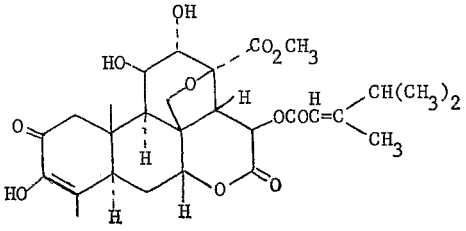
[1]For the previous part of this series see (13).

[2]Office de la Recherche Scientifique et Technique Outre-Mer, B.P. 165, Cayenne, Guyane Française.

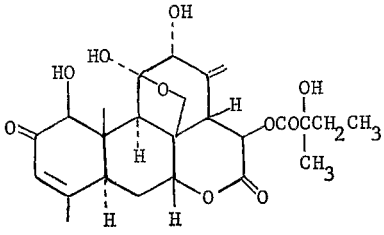
[3]Contribution 65 of the series "Antineoplastic Agents". For part 64 refer to ref. (1).

[4]Based on part of the Ph.D. dissertation submitted by SBS to the University of North Bengal, India, 1977. In the dissertation quassinoids **4a** and **3b** were provisionally named grandilactones A and B respectively.

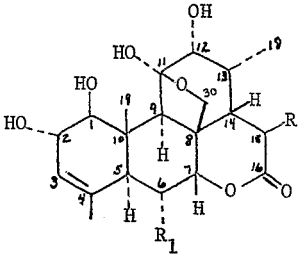
[5]The present contribution is dedicated to the memory of the late Prof. Khastgir.



1

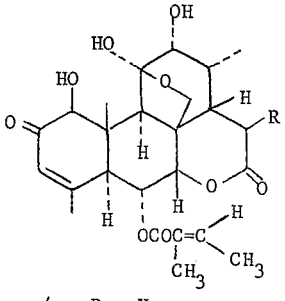


2



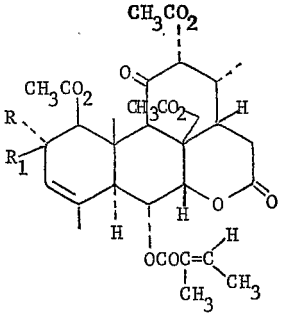
3a, R = OCOC(CH$_3$)(OAc)CH$_2$CH$_3$, R$_1$ = H

b, R = H, R$_1$ = OCOC(CH$_3$)=CHCH$_3$



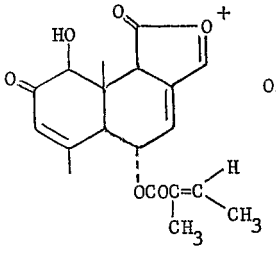
4a, R = H

b, R = OCOCH$_3$

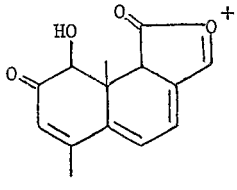


5a, R = OCOCH$_3$, R$_1$ = H

b, R = R$_1$ = O



6



7

ions at *m/e* 378 (M^+-100), 83 (C_5H_7O) and 55 (C_4H_7) and by analysis of the 250 MHz ^{1}H nmr spectrum (table I and figure 1). The latter spectrum showed signals for methyl protons at δ 1.60 (doublet, C–3$'$) and 1.89 (singlet, C–2$'$), and a quartet signal for the C–3$'$ vinyl proton at δ 7.09.

Comparison of the spectral data with that of other quassinoids such as **2** or **4a** suggested that the new substance was a quassinoid containing a vicinal glycol in ring A, instead of an α-ketol. The location and stereochemistry of the ester group in **3b** was assigned by nmr double resonance studies. Irradiation of the quartet

TABLE 1. 250-MHz ^{1}H nmr spectra[a] of quassinoids **4a**, **3b** and **5a**.

	4a[b]	**3b**[c]	**5a**[d]
H-1	4.15	4.15 d (7.6)	5.11 d (6.8)
H-2		4.61 m (7.6)	5.33 d (6.8)
H-3	6.11 br	5.86 br	5.51 br
H-6	5.58 dd (11, 2.7)	5.95 dd (12, 2.3)	5.32 dd (11, 2.6)
H-7	4.52 d (2.7)	4.80 d (2.0)	4.71 d (2.6)
H-9	2.82	3.19	3.31
$-CH_2O-$	3.76 d (9.1)	3.86 d (9)	4.04 d (12.8)
	4.17 d	4.47 d	4.63 d
H-12	3.60 d (4.2)	4.00 m (3)	5.05 d (2.7)
CH_3-13	1.04 d (6.8)	1.11 d (6.8)	1.00 d (6.4)
CH_3-10	1.38	1.86	1.55
CH_3-4	2.02	1.89	1.72 br
CH_3-2'	1.88	1.89	1.85
CH_3-3'	1.83 d (7.2)	1.60 d (6.8)	1.83 d (7.2)
H-3'	7.05 q (7.2)	7.09 q (6.8)	7.27 q (7.2)
OAc			1.82
			2.00
			2.13
			2.19

[a]Shifts in ppm and coupling constants as (Hz).
[b]Solution in deuterochloroform- 5% pyridine-d_5.
[c]Pyridine-d_5 solution.
[d]Deuterochloroform solution.

corresponding to the C-6 proton (δ 5.95) caused the signal for the adjacent proton at δ 4.80 (H-7) to become a singlet. Analogously, irradiation at δ 4.80 resulted in collapse of the C-6 proton quartet to a doublet at δ 5.95. The structure of quassinoid **3b** was further supported by interpretation of its ^{13}C nmr spectrum which showed two carbonyl resonances (δ 168.8 and 166.0), four additional sp^2 carbon atoms (δ 138.1, 133.5, 128.7 and 127.8) and seven oxygen-bearing carbon atoms at δ 109.8, 82.7, 2 x 78.7, 71.9, 69.9 and 67.8 assigned to C-11, C-1, C-12, C-7, C-2, C-30 and C-6, respectively (15).

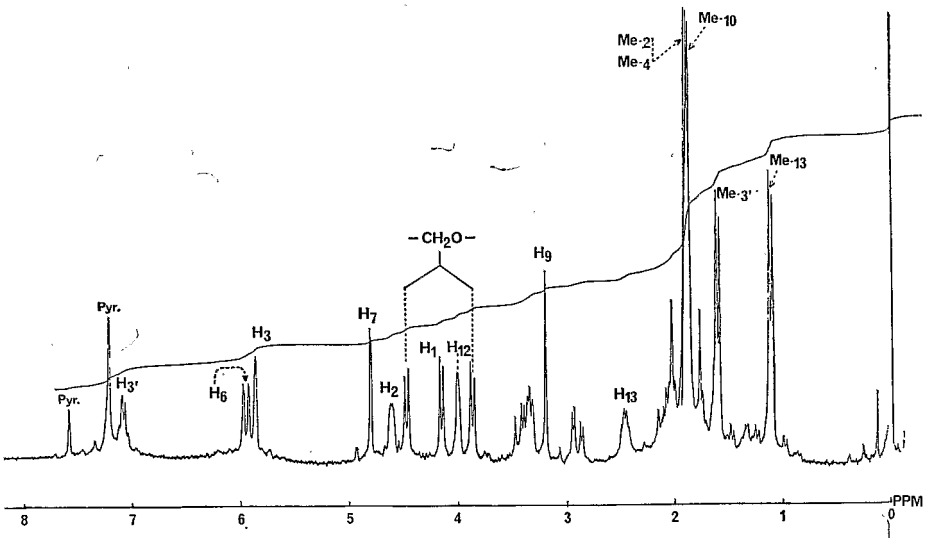


FIG. 1. 250-MHz ^{1}H nmr spectrum of 6α-tigloyloxychaparrin (**3b**) in pyridine-d_5.

In agreement with the proposed structure (**3b**), acetylation (acetic anhydride/pyridine) afforded a tetraacetate derivative (**5a**). The mass spectrum of tetraacetate (**5a**) showed a molecular ion at *m/e* 646 with significant fragmentation ions at *m/e* 586 (M^+–60), 486 (M^+–60–100) and 444 (M^+–60–100–42). The circular dichroism curve displayed a Cotton effect at 309 nm characteristic of the C–11 oxo group (16). Assignment of structure **3b** was unequivocally confirmed by chemical correlation with 6α-tigloyloxychaparrinone (**4a**). Oxidation of glycol **3b** with manganese dioxide (17) yielded 6α-tigloyloxychaparrinone (**4a**) identical with an authentic sample.[6]

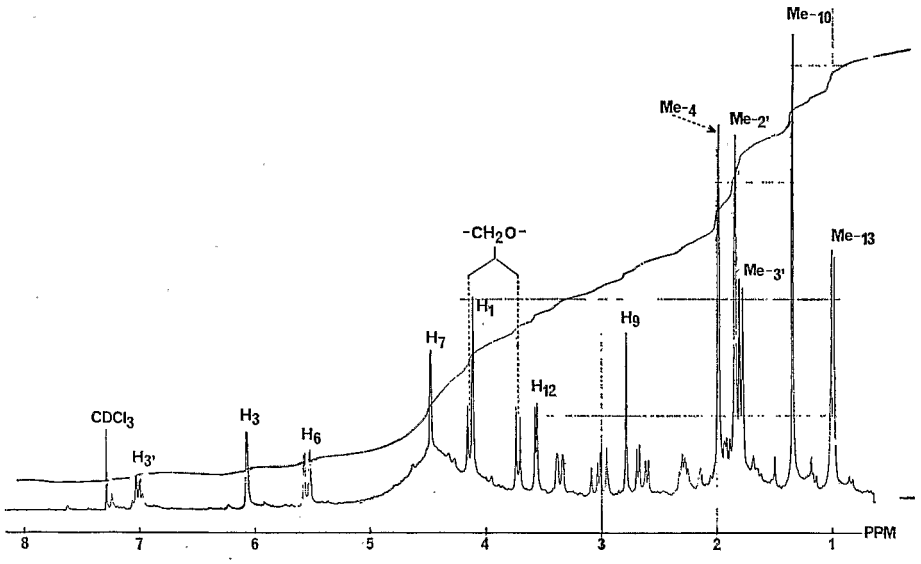


FIG. 2. 250-MHz ^1H nmr spectrum of 6α-tigloyloxychaparrinone (**4a**) in deuterochloroform containing 5% pyridine-d_5.

Careful column chromatographic (silica gel) separation of the 6α-tigloyloxychaparrin (**3b**) mother liquors led to the isolation of 6α-tigloyloxychaparrinone (**4a**). The structure of α-ketol **4a** was established by interpretation of spectral data (11) as outlined above for the glycol derivative **3b**. The mass spectrum fragment ions at *m/e* 345 (**6**) and 245 (**7**) provided further evidence for assigning the tiglate ester to position 6 (18, 19). Additional confirmation for the structural assignment was obtained by characterization of the triacetate derivative (**5b**) and by comparison with the same substance[6] isolated from *Ailanthus integrifolia* ssp. *calycina* (11).

An analogous structural investigation of two quassinoids isolated (benzene extract) from bark of the tall (and very straight) tree *Ailanthus grandis* Prain (Simaroubaceae, North Bengal, India) resulted in their characterization as glycol **3b** and α-ketol **4a**. Interestingly, the availability of quassinoids **3b** and **4a** from *A. grandis* depends rather markedly upon the collecting location. Trees growing on the plains near the foothills provided a reasonable yield of the quassinoids, but the same species at 300–500 m higher elevation was not useful for this purpose.

[6]We wish to thank Drs. N. R. Farnsworth, G. A. Cordell and A. D. Kinghorn for an authentic specimen of 6α-tigloyloxychaparrinone.

The specimens of the lactones **3b** and **4a** isolated from *A. grandis* and *S. cuspidata* were found to be mutually identical. The National Cancer Institute's murine P388 lymphocytic leukemia was employed to evaluate each lactone. With the P388 *in vitro* system (14) lactones **3b** and **4a** exhibited cell growth inhibition at the significant levels of 0.24 and <0.01 μg/ml, respectively. While glycol **3b** was found inactive in the P388 *in vivo* system (20) at dose levels up to 2.0 mg/kg, the α-ketol (**4a**) showed 34–63% increases in survival time at doses of 0.08 to 0.60 mg/kg respectively. The antineoplastic activity observed when 6α-tigloyloxychaparrinone (**4a**) was used corresponded quite well with that obtained independently by Farnsworth and colleagues (11). Apparently the diosphenol-like α-ketol carbonyl group (see **1**) of quassinoids **2** and **4a** is an important structural feature for antineoplastic activity.[7]

EXPERIMENTAL[8]

ISOLATION OF 6α-TIGLOYLOXYCHAPARRIN (**3b**) and 6α-TIGLOYLOXYCHAPARRINONE (**4a**). A. From *Simaba cuspidata*.—Dried, finely ground stem bark (1.4 kg, collected in French Guiana) was extracted with hexane and several times with hot water (80°). The aqueous extract was concentrated (*in vacuo*) and continuously extracted with chloroform. Evaporation of the chloroform yielded a bright yellow foam (3 g) which crystallized upon addition of chloroform to give 0.20 g of 6α-tigloyloxychaparrin (**3b**). Recrystallization of glycol **3b** from 95% ethanol afforded colorless needles, mp 273–275°; $[\alpha]_D$+130° (c, 0.7 in pyridine). Glycol **3b** was found relatively insoluble in a variety of organic solvents and slightly soluble in methanol, ethanol, pyridine and dimethylsulfoxide. The mass spectrum showed M^+ at *m/e* 478 and the ^1H nmr data has been entered in table 1 and figure 1; ^{13}C nmr (pyridine-d_5 solution), δ 67.8, 69.9, 71.9, 2 x 78.7, 82.7, 109.8, 127.8, 128.7, 133.5, 138.1, 166.0 and 168.8.

Anal. Calcd. for $C_{25}H_{34}O_9$: C, 62.75; H, 7.16. Found: C, 62.25; H, 7.06.

Acetylation of glycol **3b** with acetic anhydride-pyridine (24 hr, room temperature) yielded a non-crystalline tetraacetate; CD λmax 309 nm (Δε−1.13; c, 1.48 in dioxane). The tetraacetate (**5a**) corresponded to empirical formula $C_{33}H_{42}O_{13}$ with significant mass spectral ions at *m/e* 646 (M^+), 586, 486 and 444. The 250 MHz nmr data has been recorded in table 1.

The mother liquor residue from crystallization of glycol **3b** was subjected to column chromatography (silica gel 60). Elution with 95:5 chloroform-methanol afforded 6α-tigloyloxychaparrinone (**4a**, 80 mg); when the solvent ratio was changed to 9:1, additional glycol **3b** (0.12 g) was obtained. Crystallization from ethyl acetate gave α-ketol **4a** melting at 229–231° [lit. (11) mp 230–2°]; ms: *m/e* 476 (M^+) 345 (ion **6**), 245 [ion **7**], 83 (C_5H_7O), and 55 (C_4H_7); $[\alpha]_D$+156° (c, 0.81 in chloroform); and uv λmax 228 nm (ε 15,500); the 250 MHz nmr data has been entered in figure 2 and table 1. An analogous isolation study of *S. cuspidata* root bark again provided α-ketol **4a**.

B. From *Ailanthus grandis*.—Dried, powdered stem and trunk bark[9] (5 kg collected near

[7]For another recent example refer to the characterization and antineoplastic evaluation of undulatone (**4b**, Ref. 19, 21).

[8]Melting points were determined on a Kofler melting point apparatus and are uncorrected. Optical rotations were determined (room temperature) with a Perkin-Elmer model 241 or Roussel-Jouan Quick Polarimeter. Circular dichroism measurements were made with a Roussel-Jouan Dichrographe II. Infrared spectra were recorded with a Perkin-Elmer model 257 or a Beckman model 12 spectrometer. The ultraviolet spectra were measured with a Spectronic model 505 (Bausch and Lomb). Electron impact mass spectral determinations were performed with AEI model MS-50 (by Mr. C. Girard) and Varian MAT 112S spectrometers (by Miss Mary J. Cullen). The ^{13}C nmr spectrum (in ppm downfield from tetramethylsilane) was obtained with a Bruker HXE-90 (22.6 MHz) instrument by Mme. C. Fontaine. The 250 MHz, 240 MHz and 100 MHz spectra ^1H nmr were recorded respectively by Mr. C. Merienne (Cameca spectrometer), Dr. D. B. Naskar and Dr. J. Witschel, Jr. (Varian XL-100).

All solvents employed for chromatography were redistilled. Preparative tlc was performed on Whatman Linear-K silica gel plates (1000 μ thick). Analtech Uniplates (silica gel) were used for tlc. Sulfuric acid spray (gives a deep red color with some quassinoids) followed by heating (10 min) easily developed the quassinoids. Column chromatography was performed with silica gel 60 in columns and in prepacked Size B columns (both from E. Merck, Darmstadt). A Gilson UV monitor (model HM) was used to follow column progress and a microfractionator (Gilson FC 80) was employed to collect the fractions. The mutual identity of specimens was established by thin-layer chromatographic and infrared spectral (KBr) comparisons and by mixture melting points.

[9]A herbarium specimen is present in the herbarium of the Department of Botany, University of North Bengal.

North Bengal, India) was extracted (Soxhlet apparatus) with benzene[10] (20 hr). The benzene solution was concentrated to 1.2 liters, whereupon, a dark colored solid separated and was collected by filtration. The crude glycol (**3b**, 1.5 g) was recrystallized several times from methanol to provide needles melting at 278°C.

Further purification of glycol **3b** was required (indicated by tlc with chloroform-methanol-acetic acid 90:9:1). Quassinoid **3b** gave a red color on a tlc plate when sprayed with concentrated sulfuric acid and brown when heated, but quassinoid **4a** developed solely as a brown color only upon being heated. While a variety of careful column chromatographic techniques were applied to purification of glycol **3b**, the following preparative thin-layer procedure proved most convenient. The crude glycol (**3b**, 30 mg) was applied to a Whatman Linear-K preparative plate with use of methylene chloride-methanol-acetic acid (65:10:1) as mobile phase. The band at R_f=0.4 (uv absorption) yielded pure glycol **3b** (26 mg) which was recrystallized (3 times) from methanol to afford an analytical specimen of 6α-tigloyloxychaparrin, dp 274–278°; mixed melting point with **3b** from part (A) above, 272–274 (dec.); ^{1}H nmr (pyridine d_5), δ1.10 (d, J=7 Hz, 3H), 1.60 (d, J=7 Hz, 3H), 1.88 (s, 9H), 3.17 (s, 1H), 3.83; 4.45 (AB, J=9 Hz), 4.77 (d, J=2 Hz, 1H), 5.84 (bs, 1H), 5.92 (dd, J=12; 2 Hz, 1H), 7.12 (J=6 Hz, 1H); ir (KBr) 3455, 3400, 2965, 1726, 1700, 1391, 1256, 1133, 1060, 1020, 970, 735 cm^{-1}; ms *m/e* (1%) 478 (M^+, 2), 460 (2), 378 (5), 360 (10), 264 (7), 246 (20), 231 (44), 157 (14), 105 (14), 95 (22), 91 (15), 83 (24), 82 (99), 69 (24), 57 (25) and 55 (100).

Anal. Calcd for $C_{25}H_{34}O_9$: C, 62.76; H, 7.16. Found: C, 62.94; H, 7.12.

The benzene filtrate from separation of glycol **3b** was concentrated to *ca.* 100 ml and the solid that separated was collected. Several recrystallizations from methanol yielded 6α-tigloyloxychaparrinone (**4a**): mp 227–229° [lit. (11) mp 230–2°]; mixture melting point with **4a** from part (A) above, 228–230°; [α]D+195.7° (pyridine); uv λ max (methanol) 225 nm (ε 25,000); ms: *m/e* 476 (M^+), 393, 376, 345, 264, 262, 248, 247, 245, 151, 135, 83 and 55.

Anal. Calcd for $C_{25}H_{32}O_9$: C, 63.01; H, 6.77. Found: C, 62.98; H, 6.65.

To 6α-tigloyloxychaparrinone (**4a**, 0.20 g) in pyridine (4 ml) was added acetic anhydride (4 ml). The solution was heated (3 hr water bath), cooled, and poured into water (0°C); the solid which separated was collected and washed with water. Recrystallization from methanol afforded triacetate **5b**: mp 160° [lit. (11), mp 240–2°]; uv λ max (methanol) 225 nm (ε 25,500); ir (Nujol), 1740, 1725, 1700, 1675, 1640, 1615 and 1230 cm^{-1}; 240 MHz nmr, δ1.06 (d, 3H), 1.53 (s, 3H), 1.9 (m, 3H), 2.0 (m, 6H), 2.06 (s, 3H), 2.2 (s, 6H), 2.53 (m, 1H), 3.5 (s, 1H), 4.07; 4.75 (AB, J=13 Hz), 4.82 (m, 1H), 5.0 (m, 1H), 5.27 (s, 1H), 5.46 (d, 1H), 6.1 (m, 1H) and 7.1 (m, 1H); ms: *m/e* 602 (M^+), 531, 502, 460, 83 and 55.

Anal. Calcd for $C_{31}H_{38}O_{12}$: C, 61.77; H, 6.37. Found: C, 61.85; H, 6.31.

The specimens of glycol **3b** and α-ketol **4a** obtained from both *S. cuspidata* and *A. grandis* were found to be identical. Also, a sample of α-ketol **4a** from *S. cuspidata* was compared with the same substance[6] from the root bark of *A. integrifolia* (11); they were found to be mutually identical.

OXIDATION OF 6α-TIGLOYLOXYCHAPARRIN (**3b**) TO 6α-TIGLOYLOXYCHAPARRINONE (**4a**).—A dioxane (20 ml) solution of glycol **3b** (57 mg) was treated (8 hr, room temperature) with active manganese dioxide (0.5 g), (17). After column chromatographic purification and recrystallization from ethyl acetate, pure α-ketol **4a** (31 mg) was obtained. The product (**4a**) was identical with α-ketol **4a** isolated from *S. cuspidata*.

ACKNOWLEDGMENTS

The ASU–CRI group is grateful to the National Cancer Institute (performed pursuant to Contract NO1–CM–97297 with the Division of Cancer Treatment, NCI, National Institutes of Health, Department of Health, Education and Welfare), and to the National Cancer Institute, DHEW, (Grant nos. CA16049–05 and 06), the Fannie E. Rippel Foundation, Mrs. Mary Dell Pritzlaff, the Olin Foundation (Spencer T. and Ann W.), Mr. Jack Whiteman and the Phoenix Coca Cola Bottling Co. for partial support of this investigation.

Received 19 November 1979.

LITERATURE CITED

1. G. R. Pettit, D. P. Gieschen and W. E. Pettit, *J. Pharm. Sci.*, **68**, 1539 (1979).
2. J. D. Hooker, "Flora of British India," Vol. I, 1973, p. 517.
3. J. F. Morton, "Major Medicinal Plants," C. C. Thomas, Springfield, Illinois, 1977, p. 365.
4. K. R. Kirtikar and B. D. Basu, "Indian Medicinal Plants," Vol. I, 1975, pp. 503–504.
5. J. Polonsky, *Fortschr. Chim. Org. Naturst.*, **30**, 101 (1973).
6. M. E. Wall and M. Wani, *Abstr. Int. Symp. Chem. Nat. Prod.*, 7th, *E138*, 614 (1970).
7. S. M. Kupchan, R. W. Britton, J. A. Lacadie, M. F. Ziegler and C. W. Siegel, *J. Org. Chem.*, **40**, 648 (1975).
8. G. R. Pettit, "Biosynthetic Products for Cancer Chemotherapy," Vol. 1, p. 79, Plenum Publishing Corp., New York, N.Y., 1977.

[10]Caution: benzene is now known to be a human carcinogen.

9. M. C. Wani, H. L. Taylor, J. B. Thompson and M. E. Wall, *Lloydia*, **41**, 578 (1978).
10. J. Polonsky, Z. Varon, H. Jacquemin and G. R. Pettit, *Experientia*, **34**, 1122 (1978).
11. A. A. Seida, A. D. Kinghorn, G. A. Cordell and N. R. Farnsworth, *Lloydia*, **41**, 584 (1978).
12. J. Polonsky, Z. Varon and E. Soler, *Compt. Rend. Ac. Fr., Ser. C*, **288**, 269 (1979).
13. J. Polonsky, M. V. Tri, T. Prangé, Cl. Pascard and T. Sevenet, *J. Chem. Soc., Chem. Comm.*, 641 (1979).
14. G. R. Pettit, C. L. Herald, G. F. Judd, G. Bolliger and P. S. Thayer, *J. Pharm. Sci.*, **64**, 2023 (1975); and J. M. Schmidt and G. R. Pettit, *Experientia*, **34**, 659 (1978).
15. J. Polonsky, Z. Baskevitch, H. E. Gottlieb, E. W. Hagaman and E. Wenkert, *J. Org. Chem.*, **40**, 2499 (1975).
16. J. Polonsky, Cl. Fouquey and A. Gaudemer, *Bull. Soc. Chim. Fr.*, 1827 (1964).
17. O. Mangera, G. Rosenkranz and F. Sondheimer, *J. Chem. Soc.*, 2189 (1953).
18. J. L. Fourrey, B. C. Das and J. Polonsky, *Org. Mass Spectrom.*, **1**, 819 (1968).
19. N. Bourguinon-Zylber, Doctoral Thesis, Orsay, France, 1968.
20. R. I. Geran, N. H. Greenberg, M. M. MacDonald, A. M. Schumacher and B. J. Abbott, *Cancer Chemother. Rep.*, Part 3, 3, N 2 (1972).
21. M. C. Wani, H. L. Taylor, J. B. Thompson, M. E. Wall, A. T. McPhail and K. D. Onan, *Tetrahedron*, **33**, 17 (1979).