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COUMARIN CONSTITUENTS OF *HERACLEUM CANDICANS*—III

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Abstract—8-Geranoxypsoralen was isolated from *H. candicans* and characterized by degradations and synthesis.

IN PREVIOUS communications the isolation of two furocoumarins heraclenin[1] and heraclenol,[2] from *Heracleum candicans* roots was reported. The former compound was obtained when the crude coumarin mixture from the petroleum ether extract of the plant roots was chromatographed over deactivated alumina[3] using petroleum ether–benzene (1:1) for elution, and was found to be the major coumarin constituent of the plant. Evaporation of the petroleum ether and petroleum ether–benzene (5:1) eluates also gave two other compounds in small amounts which crystallized well and had m.ps (A) 53–54° and (B) 82–83° respectively. Heraclenol mentioned above is not present in the petroleum ether extract and was obtained on subsequent extraction of the roots with benzene.

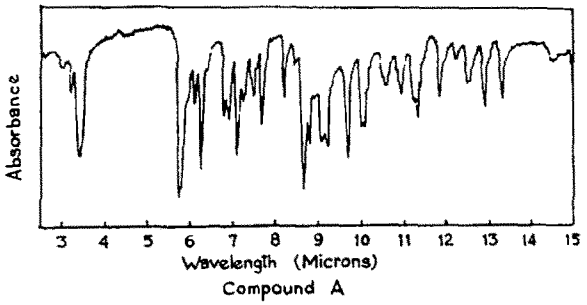
The furocoumarin nature of the two compounds was indicated by three strong absorption maxima in their UV spectra.[4] IR spectra of both A and B showed absence of free hydroxy groups and the presence of a 6-membered-α,β-unsaturated lactone carbonyl. Acid catalysed degradation of compound A gave xanthotoxol and geraniol. This suggested that the compound was 8-geranoxypsoralen reported earlier.[5] However as the same degradation products were obtained from compound B, it was suspected that one or the other of these compounds was either a mixture or a geometrical isomer which could only mean that it was neryl instead of geranyl ether. Both compounds gave single fluorescent spots on chromatostrips[6] having identical R_f values, but analytical values of compound B did not agree with those required for 8-geranoxypsoralen.

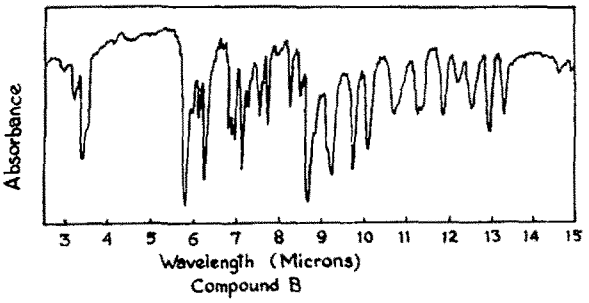
Comparison of the two products with a sample of 8-geranoxypsoralen kindly sent by Dr. W. L. Stanley established the identity of compound A. The sample supplied melted at 53–54°, as against 61–62° reported by Stanley *et al.* who had assigned the 8-geranoxypsoralen structure merely on the basis of acid degradation to xanthotoxol and an indication of the presence of a C_{10} side chain from the analytical values. They failed to isolate any leavulinic aldehyde[7] on ozonolysis. The isolation of two

[1] Y. N. Sharma, A. Zaman and A. R. Kidwai, *Tetrahedron* **20**, 87 (1964).
[2] Y. N. Sharma, R. C. Sharma, A. Zaman and A. R. Kidwai, *Naturwissenschaften* **51**, 537 (1964).
[3] P. Crabbe, P. P. Leming and C. Djerassi, *J. Amer. Chem. Soc.* **80**, 5258 (1958).
[4] D. P. Chakraborty and S. K. Chaudhary, *Trans. Bose Res. Inst.* **24**, 15 (1961).
[5] W. L. Stanley and S. H. Vannier, *J. Amer. Chem. Soc.* **79**, 3488 (1957).
[6] J. M. Miller, J. G. Krichner and G. J. Keller, *Anal. Chem.* **23**, 420 (1951).
[7] W. L. Stanley, *Proc. Third Ann. Symp. P.P.G.N.A. Toronto*, 79 (1963).

compounds having different m.ps but giving identical cleavage products made reinvestigation of the side chain necessary.

IR spectra of the two compounds differed only in the presence of an extra band at 10·6 μ in the spectrum of compound A. The UV spectra were also identical. Ozonolysis of both compounds gave leavulinic aldehyde and acetone which were identified as 2,4-dinitrophenylhydrazone derivatives, thus excluding any difference in the attachment of the side chain to the coumarin residue. This left only the possibility



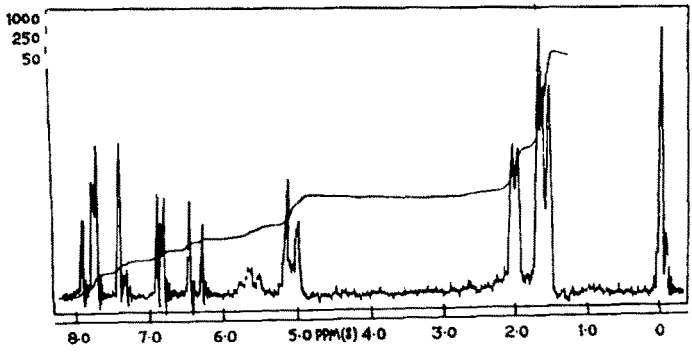


FIGS 1 and 2. IR spectra.

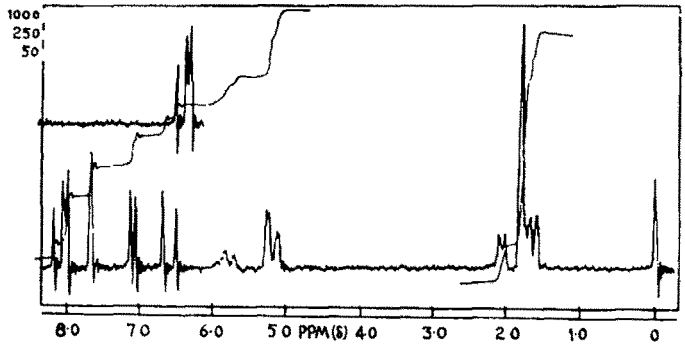
of compound B being either the *cis* isomer or contaminated with some other impurity.

Recently the nature of the side chain in mycelianamide was reinvestigated[8] and the same methods were applied in this case. The stereochemistry of the side chain was established by Birch reduction. VPC analysis of the product obtained from the reduction of compound B showed peaks attributed to *trans*-2,6-dimethyl-2,6-octadiene (methylgeraniolene) and 2-methyl-2-butene, thereby indicating it to be a mixture of imperatorin and 8-geranoxypsoralen. Sufficient material was not available for a similar degradation of compound A, but the presence of a geranyl side chain here also could be established by comparison with synthetic material. The problem of difference in the m.ps of these two compounds was settled by comparison of their NMR spectra. A doublet at 4·82 τ due to —O—CH_2—C=C— occurs in both C_5 and C_{10} compounds while the absorption at 7·91 τ is due to —C=C—CH_2—CH_2—C=C— which occurs only in the C_{10} compound. The integrated intensities of compound B show a 2 to 1 ratio in favour of the —O—CH_2—C=C protons. Thus the side chain must consist of 3 times as much C_5 as C_{10}. This along with the above degradations suggested that it was a mixture of 8-geranoxypsoralen and imperatorin in the ratio of

[8] R. B. Bates, J. H. Schauble and M. Soucek, *Tetrahedron Letters* No. 25, 1683 (1963).



Compound A



Compound B

FIGS 3 and 4. NMR spectra.

1:3. This is also in agreement with the analytical values. Repeated attempts to separate the two compounds on chromatostrips on various adsorbents were unsuccessful. It was found that authentic 8-geranoxypsoralen when mixed with imperatorin in about the same proportion could not be separated on chromatostrips or on paper chromatograms.

An attempt to synthesize 8-geranoxypsoralen using the procedure of Chatterjee and Chaudhary[9] for the synthesis of bergamottin did not succeed. It could, however, be synthesized by the method of Schönberg and Sina.[10] The product gave no depression in mixed m.p. with the natural sample.

EXPERIMENTAL

All UV spectra (Beckmann model DU instrument in 95% EtOH); IR spectra (Perkin–Elmer Infracord either in $CHCl_3$ soln or as mulls in nujol) and NMR spectra (Varian A-60 spectrometer).

Isolation. The crude mixture of coumarins (5 g) obtained from the pet. ether extract of *Heracleum candicans* roots (80 g) was chromatographed over AcOH-deactivated alumina, with pet. ether to give 100 mg of a low melting semisolid, sparingly soluble in pet. ether. Repeated crystallizations from a large excess of this solvent afforded stout needles (compound A), m.p. 53–54°, λ_{max} 215 mμ (log ε 4·51) 248 mμ (log ε 4·42) and 298 mμ (log ε 4·13). (Found: C, 74·32; H, 6·73. Calc. for $C_{21}H_{24}O_4$: C, 74·09; H, 7·11%.)

[9] A. Chatterjee and B. Chaudhary, *J. Chem. Soc.* 2246 (1961).

[10] A. Schönberg and A. Sina, *J. Amer. Chem. Soc.* 72, 4862 (1950).

Continued elution of the column with pet. ether benzene (5:1) gave 500 mg of a solid insoluble in pet. ether, which crystallized from MeOH in needles (compound B), m.p. 82–83°, λ_{max} 217 mμ (log ε 4·47), 248 mμ (log ε 4·42) and 298 mμ (log ε 4·05).

Thin layer chromatography. Chromatostrips were prepared according to Krichner and Miller using alumina (E. Merk) containing 2% starch. Both the products gave one fluorescent spot having identical R_f. Chromatostrips were developed with pet. ether–benzene (7:3).

Acetic acid cleavage. Compd A (2 g) was treated with glacial AcOH (2 ml) in an oil bath at 115–120° for 1½ hr, and then left overnight at room temp when a solid separated out. This was extracted thrice with 50 ml portions of hexane and the combined extract was washed with water and Na_2CO_3 to remove AcOH. The hexane extract on evaporation left a sweet smelling oil which was hydrolysed directly with 10% MeOH–KOH to give 200 mg geraniol. (Found: C, 78·56; H, 11·92. Calc. $C_{10}H_{18}O$: C, 77·86; H, 11·76%.)

The residue after the extraction of the reaction products with hexane was crystallized from ether–hexane to give light yellow crystalline mass, identified as xanthotoxol. (Found: C, 64·98; H, 3·14. Calc. $C_{11}H_6O_4$: C, 65·35; H, 2·99%.)

Xanthotoxol acetate. Xanthotoxol (500 mg), Ac_2O (5 ml) and fused AcONa (100 mg) were refluxed for 1 hr. The mixture was cooled, poured over crushed ice and the acetate crystallized from MeOH, m.p. 178°. (Found: C, 64·88; H, 3·51. Calc. $C_{13}H_8O_5$: C, 63·94; H, 3·30%.)

Xanthotoxin. Xanthotoxol (500 mg) in MeOH (5 ml) was treated with excess diazomethane in ether and left overnight. The residue was dissolved in benzene and chromatographed over AcOH-deactivated alumina, elution with benzene gave a product, m.p. 146–147°, after crystallization from benzene–pet. ether.

Xanthotoxin nitrate. Xanthotoxin (100 mg) in glacial AcOH (5 ml) was treated with HNO_3 (sp. gr. 1·42; 1 ml). The reaction mixture after standing at room temp for 2 hr and dilution with water gave a bright yellow solid, m.p. 236° (MeOH).

3,5-*Dinitrobenzoate of geraniol.* The oil obtained as above (100 mg) in dry benzene (10 ml) was treated with a solution of 3,5-dinitrobenzoyl chloride (200 mg) in dry benzene (10 ml) with the addition of 2 drops of pyridine and the mixture heated on a water bath for 20 min. The reaction mixture was diluted with excess ether and the ether–benzene layer washed thoroughly with cold water, dried (Na_2SO_4) and the solvent removed *in vacuo*. The residue was crystallized from MeOH, m.p. and m.m.p. 62–63°.

Ozonolysis of compd A. A solution of compd A (500 mg) in glacial AcOH (20 ml) was treated with ozonized oxygen for 2 hr. Water (30 ml) and Zn dust (100 mg) were then added and the mixture warmed on a water bath till it became clear. After dilution with a further quantity of water (70 ml) the solution was steam distilled and the distillate collected directly in fractions in 2N H_2SO_4 solution of 2,4-dinitrophenylhydrazine. The first 10 ml of distillate yielded a bulky orange precipitate which dissolved completely in hot MeOH. The filtered solution on cooling deposited orange plates of acetone-2,4-dinitrophenylhydrazone, m.p. and m.m.p. 123–124°. Later fractions of the steam distillate gave a bright yellow derivative which was crystallized from nitrobenzene–EtOH to give a powder identified as laevulinic aldehyde, m.p. and m.m.p. 233–234°.

Birch reduction of compd B. A three-necked 500 ml round bottom flask was fitted with a dry ice condenser, stirrer and a dropping funnel. Ammonia (75 ml) was condensed in the flask and Na metal (3 mg) was added in small pieces, producing a blue solution. To the vigorously stirring Na–NH_3 solution under an N_2 atm compound B (500 mg) in 20 ml MeOH was added dropwise over ½ hr. As the blue colour of Na–NH_3 disappeared so more Na was added to maintain the colour for 1 hr. Then heptane (25 ml), granulated NH_4Cl (10 g) and water (50 ml) were added to the refluxing reaction mixture. After separating the heptane layer, the aqueous layer was washed with three 25 ml portions of heptane. The combined heptane extracts were washed with water, until the washings were neutral, and dried ($MgSO_4$). The VPC analysis of the product showed peaks attributed to 2-methyl-2-butene and *trans*-2,6-dimethyl-2,6-octadiene.

Geranyl bromide. PBr_3 (12 g) in pet. ether (40–60°) (10 ml) was added dropwise during 1 hr at −7° to geraniol (15 g) in pet. ether (29 ml) and pyridine (2·5 ml) with constant stirring. The reaction mixture was further stirred for 20 min at −7°, poured over crushed ice, stirred for 15 min and then treated with pet. ether (100 ml). The pet. ether layer was separated, washed twice with water and once with dil. $NaHCO_3$aq dried (Na_2SO_4) and the solvent evaporated. The residue was distilled at 102–103°/3 mm.

Condensation of xanthotoxol and geranyl bromide. A mixture of xanthotoxol (0·2 g), anhydrous K_2CO_3 (3 g), anhydrous acetone (50 ml) and geranyl bromide (2 ml) was refluxed for 36 hr. The acetone soln was filtered and the residue washed 5 times with 20 ml portions of dry acetone. The acetone washings were combined and the solvent removed. The residue was dissolved in a minimum amount of dry benzene and chromatographed over AcOH-deactivated alumina. Elution with pet. ether gave a viscous mass, which crystallized from pet. ether in needles m.p. and m.m.p. 53–54°. The IR spectra of natural and synthetic products were superimposable.

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