Disparolone, a Novel Tumor Inhibitor Derived from the Gypsy Moth *Portheria dispar* (L.)

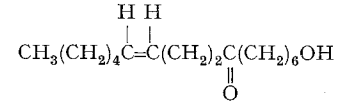
Incidental to our continuing search for insect attractants from plant and insect products, we have submitted extracts and fractions of such natural products to Drug Research and Development, National Cancer Institute, Bethesda, Maryland, for tests as tumor inhibitors. During the extraction and isolation of sex attractant from abdomens of female gypsy moths, *Porthetria dispar* (L.)[1] large amounts of both combined and free fatty acids were obtained that were unattractive to the male moths. BUTENANDT[2], in his investigation of the sex attractant of the silkworm moth, *Bombyx mori* L., had found that reduction of the unattractive acids to the corresponding alcohols with lithium aluminum hydride resulted in active compounds. Such reduction of our gypsy moth acids failed to give products attractive to gypsy moth males, but the crude reduction mixtures showed in vivo inhibitory activity against the Walker intramuscular carcinosarcoma 256 (WA) in rats[3]. We have now succeeded in isolating and identifying one of the major cytotoxic principles, and its structure has been confirmed by synthesis.

Initially, the combined fatty acids (52 g) obtained from 100,000 female moths[1] were reduced with 7 g of lithium aluminum hydride in ether to give 49 g of a viscous, yellow oil (n_D^{25} 1.4601) that solidified in the cold but melted again at room temperature. This oil showed inhibition of the tumor system at 400 mg/kg. Guided by the assay, we then fractionated the oil on a column (64 by 3 cm) of silicic acid (minus 325 mesh), eluting successively with 10-, 20-, and 30% benzene in hexane, 2-, 8-, 20-, and 50% ethyl ether in hexane, ether, 5- and 10% methanol in ether, and methanol. Inhibition at 300 mg/kg was demonstrated by the 10% methanol in ether eluate, which weighed 1.90 g after evaporation of the solvent. The remaining fractions showed no inhibition of the tumor system. The active fraction was a very viscous, pale-yellow oil at room temperature, but it solidified when refrigerated (5 °C) to a white, waxy solid, m.p. 25 °C.

Gas-liquid chromatography of the fraction melting at 25 °C on polar and non-polar column packings showed it to be a pure compound. Elemental analysis established its formula as $C_{16}H_{30}O_2$. The IR-spectrum exhibited strong bands for primary hydroxyl (3400 and 1070 cm^{-1}) and ketone carbonyl (1720 cm^{-1}), weak bands for unsaturation (1660 cm^{-1}), and an unbroken chain of at least 4 methylene groups (718 cm^{-1}); absence of branching was likewise indicated. The UV-spectrum showed only end absorption, which precluded the presence of conjugation. The fact that the compound contained a keto group showed that lithium aluminum hydride reduction of the acids had been incomplete, probably because of the use of insufficient reducing agent. This explanation was substantiated by further reducing the keto alcohol with excess lithium aluminum hydride to diol, as shown by disappearance of the carbonyl band from its IR-spectrum.

The keto alcohol, which has been designated 'disparolone', failed to give a positive iodoform test and was therefore not a methyl ketone. Hydrogenation in the presence of platinum catalyst showed the absorption of sufficient hydrogen for a single double bond, gave a compound that analyzed for $C_{16}H_{32}O_2$, and indicated a *cis* configuration for the double bond, since the IR-spectrum of disparolone was free of absorption for *trans* (965 cm^{-1}) and vinyl (3095–3010 and 1050–950 cm^{-1}) unsaturation. Periodate-permanganate oxidation[4] of disparolone cleaved the double bond but left the primary hydroxyl and keto groups intact to yield hexanoic acid (identified by gas chromatography of its methyl ester) and a hydroxy keto acid ($C_{10}H_{18}O_4$) (strong IR-bands at 3400, 1720, and 1710 cm^{-1}); the latter could not be a β-keto acid since it failed to give a color test with ferric chloride reagent. The *cis* double bond therefore lay between carbons 10 and 11. Sodium borohydride selectively reduced the keto group of the hydroxy keto acid to give a hydroxy lactone ($C_{10}H_{18}O_3$) instead of the expected hydroxy acid, indication that the keto group, prior to reduction, had been in the *gamma* or *delta* position. The IR-spectrum of the hydroxy lactone revealed absorption at 1770 cm^{-1}, characteristic of a γ-lactone; a Δ-lactone absorbs at 1740 cm^{-1}. Thus, the hydroxy lactone appeared to be the γ-lactone of 4,10-dihydroxydecanoic acid.

The foregoing data indicated that disparolone was (*Z*)-7-oxo-10-hexadecen-1-ol (I), a structure borne out by its nuclear magnetic resonance spectrum, and this assignment has been confirmed by synthesis.



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Disparolone was synthesized by preparing 7-oxo-10-hexadecyn-1-ol, m.p. 41–42 °C, according to the elegant 8-step method of KENNEDY et al.[5] and selectively hydrogenating it with Lindlar catalyst. The product melted at 25 °C, undepressed by admixture with disparolone, and both showed identical IR-, gas chromatographic, and NMR-spectra.

Although the oncolytic activity of disparolone is relatively low, it does provide a lead for the preparation of related compounds possibly possessing considerably higher activity. To our knowledge, anti-tumor activity has never before been reported for a long-chain keto alcohol. The most closely-related structure with such activity is 10-hydroxy-2-decenoic acid, which is active against leukemia and ascitic tumors[6].

Zusammenfassung. Disparolon, als neue tumorhemmende Verbindung von Weibchen des Schwammspinners, *Porthetria dispar* L., wurde isoliert und als 7-Oxohexadec-*cis*-10-enol-(1) identifiziert und synthetisch dargestellt. Die Verbindung ist gegen Walker Karzinosarkom 256 in Ratten aktiv.

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[7] We thank the late E. L. GOODEN for providing the nuclear magnetic resonance spectra.