PLANT INVESTIGATIONS II. STUDIES ON THE HEXANE EXTRACT OF *CIRSIUM ARVENSE*

DAVID M. PIATAK AND LARRY S. EICHMEIER
Department of Chemistry, Northern Illinois University, De Kalb, Illinois 60115

ABSTRACT.—The hexane extract of *Cirsium arvense* was investigated by application of column, thin layer, and gas chromatography and spectroscopic methods. A C_{18}-C_{31} alkane mixture, B-amyrin, taraxasterol, taraxasterol acetate, tetracosanol, hexacosanol, and octacosanol were positively identified.

Cirsium arvense, or Canadian thistle, the curse of the Midwestern farmer, is a plant which has received little attention by the natural product chemist (Shelyuta et al., 1970). Throughout the chemical literature the multitude of articles about it has centered upon its eradication although there have been scattered reports on its potential as a supply of blood coagulants (Mazzanti, 1954), seed growth inhibitors (Helgeson and Konzak, 1950), and meat-curing solution additives (Jensen and Hess, 1951). The little phytochemical work done with the *Cirsium* genus has been concerned only with the isolation of flavanoids (Wagner et al., 1960; Nakaoki and Morita, 1959; Ibid., 1960; Morita and Shimizu, 1963) and the detection of alkaloids, tannins, and flavanoids (Ismailov, 1958; Bandyukova and Shinkarenko, 1965; Kolodziejshi, et al., 1966).

In this paper we describe our preliminary investigations on the alkane, n-alcohol, and terpene components of *Cirsium arvense*.

EXPERIMENTAL

General—Melting points (mp) were taken on a Fisher-Johns apparatus and are corrected. Infrared spectra were recorded with a Beckmann IR-8, nuclear magnetic resonance spectra (n.m.r.), with a Varian A-60 on $CDCl_3$ solutions, and mass spectra with a Perkin-Elmer-Hitachi RMU-6E instrument. Silica gel HF_{254} (Merck) at a thickness of 0.5 mm was used for all thin layer chromatography (TLC). A Varian Aerograph Series 200 instrument with a flame ionization detector was used for gas liquid chromatography (GLC). The instrument was utilized at an injector temperature of 300°, a detector temperature of 325°, and a nitrogen rate of 80% flow at 65 psi. A 5 ft. by 1/8 in. SS column packed with 5% SE-30 on acid-washed dmcs 60/80 chromosorb W was used for all separations.

Preliminary Separation—The aerial portions of *C. arvense* were collected during June 1968 in DeKalb. The air-dried material (2 kg) was powdered and then exhaustively extracted with hexane in a Soxhlet apparatus. Removal of the hexane gave a residue (89 g) which was treated in 400 ml. of tetrahydrofuran with 120 ml. of 10% KOH. After heating on the steam bath for 15 minutes the resulting solution was diluted with water, and the organic materials were recovered with ether. The residue (63 g.) was then dissolved in hexane and the hexane removed *in vacuo*. This process was repeated twice more to ensure full removal of all other solvents.

The treated extract was dissolved in hexane and chromatographed on 900 g of Alcoa F-20 alumina which had been neutralized to Activity II-III by stirring it under hexane for 3 days with 27 ml. of 10% acetic acid. The column was eluted with 1.5 liter fractions. The solvent used and the fractions are as fol-

lows: hexane 1-8, 2% benzene-hexane 9-11, 5% benzene-hexane 12-14, 10% benzene-hexane 15-17, 25% benzene-hexane 18-48, 35% benzene-hexane 49-59, 50% benzene-hexane 60-68, benzene 69-75, 1% chloroform-benzene 76-80. The chromatogram had been continued with other solvents but no identifiable material was isolated.

Taraxasterol Acetate—A white solid which was shown by TLC analysis to be essentially one compound was found in Fractions 5-9. The material was chromatographed preparatively on three TLC plates with benzene. Recrystallization of the recovered material (400 mg.) from methylene chloride gave colorless crystals, mp 220-225°; $[\alpha]_D^{22}$, 96.7° (C,0.2); max(in KBr) 1735, 1642, 898 cm-¹; n.m.r. 278 (d,2H,J = 1.0), 122(s,3H), 63, 61, 58, 57, 51 c.p.s.; m/e 468(M+), 453, 408, 399, 393, 357, 339, 297, 249, 218, 205, 204, 203, 191, 190, 189 (base).

Anal. Calcd. for $C_{32}H_{52}O_2$: C, 81.99; H, 11.18. Found: C, 81.70; H, 11.12.

Reported mp and $[\alpha]_D$ values for taraxasterol acetate have been 234-248° and 95.0° (Kasprzyk and Pyrek, 1968); 238-240° and 96.0° (Chaudbury and Ghosh, 1970); 256-257° and 100° (Simonsen and Ross, 1957).

n-Alcohols—Fractions 20 (410 mg) and 21 (430 mg) were purified by treatment with Norit A. Crystallization of each from benzene-methanol yielded material melting at 75-77.5° and 80-82°, respectively. GLC analysis of both at a column temperature of 253° gave peaks corresponding to 1-tetracosanol at 146 sec., 1-hexacosanol at 238 sec., and 1-octacosanol at 394 sec. Both fractions consisted mainly of 1-octacosanol and were almost identical in alcohol proportions. Identification of latter two alcohols was made by comparing their GLC behavior to a commercial sample of 1-hexacosanol and the 1-octacosanol sample obtained in the first paper of this series (Piatak and Reimann, 1970). The 1-tetracosanol structure was inferred from the retention time pattern.

Taraxasterol—By TLC analysis fractions 23-48(7.0g.) were found to contain one compound. Recrystallization of the material from benzene, acetone, and chloroform-methanol after Norit A treatment gave 1.1 g. of colorless crystals, mp 203-205° (normal) and 213-215° (evacuated capillary); $[\alpha]^{22}D$ 90.9° (C,3.0); ν max (in KBr) 3400, 1642, 885 cm-¹; n.m.r. 277 (d,2H, J = 2.00), 64, 61, 58, 56, 51, 46 c.p.s.; m/c 426(M+), 411, 408, 357, 315, 218, 207, 189 (base).

Anal. Calcd. for $C_{30}HO_{50}$: C, 84.44; 11.81. Found: C, 84.37; H, 11.76.

Literature values for the melting point and specific rotation of taraxasterol were 213-219° and 82.0° (Kasprzyk and Pyrek, 1968); 222-225° and 93.5° (Atherinos et al., 1962).

The taraxasterol was converted to an acetate which was identical to the taraxasterol acetate obtained above and to a benzoate, mp 230-232 [reported mp 242-244° (Simpson, 1944)].

β-Amyrin—TLC analysis was used to determine that fractions 49-59 (1.5 g.), 60-68(1.2 g.), and 69-80 (0.9 g.) contained taraxasterol and another major component. The two were separated by preparative TLC using 25% ethyl acetate-hexane. GLC of the new compound at a column temperature of 235° revealed that the material consisted of two closely related compounds with retention times of 954 sec. and 1096 sec. The latter compound was identified as β -amyrin by comparison of its retention time with authentic material and by noting an

increase in the amount of response for the second peak when authentic material was added to the mixture. The more mobile material was not investigated further when comparison of its GLC characteristics to several triterpenes failed to identify it.

n-*Alkanes*—Fractions 1-4 (24g.) were rechromatographed on 720 g of Woelm neutral alumina of Activity I. The first three fractions eluted (3 x 500 ml. hexane) were combined to give 500 mg. of semicrystalline material, ^{max} no C=O absorption. GLC analysis with a programmed column temperature of 90-280° at a rate of 4-6°/min indicated the presence of 18 separate substances.

The mixture was dissolved in benzene and treated with a drop of bromine. Reanalysis of the material after removal of the solvent showed 15 substances. By comparing the remaining peaks to a set of standards identified by mass spectral analysis of gas chromatographically separated material (Reimann, 1970) and extrapolation of the regularity in the retention times, the alkanes were identified. The mixture was found to consist of the following n-alkanes: C_{18}(4.4%), C_{19}(<1), C_{20} (<1), C_{21}(<1), C_{22}(<1), C_{23}(5.8), C_{24}(1.0), C_{25}(4.1), C_{26}(1.9), C_{27}(25.3), C_{28}(2.8), C_{29}(37.8), C_{30}(1.7), C_{31} (12.4).

Discussion

Our work with *Cirsium arvense* appears to be the first report concerned with the identification of individual compounds in this species. Although Shelyuta et al. (1970) have explored this plant phytochemically, they had only concerned themselves with the classes of compounds present or not present.

Thus far, we have identified several known compounds. The first compound isolated was taraxasterol acetate, a derivative of a pentacyclic triterpene commonly found throughout the plant world. In fact, taraxasterol seems to be the major pentacyclic triterpene in *C. arvense*, since it was detected in a number of the chromatography fractions as the free alcohol or its acetate.

Taraxasterol itself appeared much further along in the column chromatography fractions. It was not readily identified at first even though it gave a single peak in GLC analysis, because the mp and specific rotation data were widely divergent from literature values. However, spectroscopic data did point to taraxasterol or a similar triterpene since an exocyclic methylene group was indicated by n.m.r. and infrared spectra, a hydroxyl group by an infrared spectrum, and a pentacyclic skeleton by a mass spectrum. Some derivatives were prepared, but the physical constants could not be fully reconciled with literature values.

The structure was finally resolved by a comparison of taraxasterol acetate with samples provided by other workers in India and Poland. Although a mixture m.p. with these samples had the same value as our material, the mass spectra and GLC characteristics of each were in full agreement, thereby fully establishing the structure.

The taraxasterol acetate isolated from the column appears to be an artifact produced by the action of acetic acid used to neutralize the column alumina on taraxasterol. Normally, acetates of triterpenes are not found readily in any quantity in plants. Since we did hydrolize the plant extract with base before chromatography, it is even less likely the acetate was originally present.

The isolation and identification of the mixture of straight chain primary alcohols obtained from the column after taraxasterol acetate

was easily accomplished by various chromatographic techniques. Although the fractions were mixtures of practically the same composition, the difference in their mp's and the sharpness of the mp's were not totally unexpected. In the first paper of this series (Piatak and Reimann, 1970) it had been found that mp's of alcohols were not satisfactory criteria for identification and that GLC comparison to material fully established by a mass spectrum was best.

A second pentacyclic triterpene, β-amyrin, was also uncovered in this plant. This terpene, however, could not be isolated owing to the presence of another compound. Although several different methods, e.g. silver nitrate impregnated TLC plates and double-dip TLC techniques, were attempted, a clear cut separation between the two could not be gotten. The -amyrin nevertheless was verified by the standard GLC technique of adding authentic material to the chromatographic sample and observing an increase in the response for that material.

The last series of compounds to be investigated were the n-alkanes. Even though these compounds were contained in the first four column fractions, they were mixed with several other materials. A second column chromatography employing a larger alumina to material ratio did give a colorless, crystalline mixture of alkanes containing a few unsaturated compounds. The unsaturated hydrocarbons could be removed readily by bromine treatment. Since the bp of the bromo addition product would be quite high, the net result of the bromine treatment is a removal of all unsaturated compounds. Of course, the peaks obtained for each alkane must be examined carefully to see if any have been increased by the addition products; but, thus far, this has not been our experience.

Acknowledgements

We wish to thank the National Science Foundation (GB7424) and the Seiffert Trust administered by the Illinois Division-American Cancer Society for partial support of this work. The assistance of K. Nusbaum and L. Neas, N.S.F. Student Science Training Program participants, in the initial stages of this project is greatly appreciated. We wish to thank Dr. S. K. Talapatra, Calcutta University, and Dr. J. Kasprzyk, University of Warsaw, for supplying samples of taraxasterol acetate.

Literature Cited

Atherinos, A. E., I. E. El-Kholy, and G. Soliman. 1962. Chemical Investigation of *Cynara scolymus* L. Part I. The steroids of the Receptacles and Flowers. J. Chem. Soc.:1700-1704.

Bandyukova, V. A., and A. L. Shinkarenko, 1965. Paper chromatographic determination of flavanoids in high altitude plants of the Teberda Reservation. Farmatsevt Zh., 20:37-41.

Chaudhury, N. A., and D. Ghosh. 1970. Taraxasterol and other triterpenoids in *Capparis sepiaria* leaves. Phytochemistry, 9, 1885.

Helgeson, E. A., and R. Konzak. 1950. Phytotoxic effects of aqueous extracts of field bindweed and of Canada thistle. N. Dakota Agr. Expt. Sta. Bull., 12: 71-76.

Ismailov, N. M. 1958. Alkaloid containing plants from the Khaldan and Agdash regions. (Boz Dagrange) Azerbaidzhan S.S.R. Izvest. Ahad. Nauk Azerbaidzhan S.S.R., Ser. Biol. i Sel' skokhoz:11-16.

Jensen, L. B. and W. R. Hess. 1951. Meat-curing pickle solutions containing antibiotics. United States patent 2,550,253.

Kasprzyk, Z. and J. Pyrek. 1968. Triterpenic alcohols of *Calendula officinalis* L. flowers. Phytochemistry, 7:1631-1639.

Kolodziejski, J. A. Mruk-Luczkiewicz, and D. Pietrzok 1966. Composition of *Cirsium oleraceum*. Farm. Polska, 22: 87-90.

Mazzanti, L. 1954. Antihemorrhagic action of *Cirsium arvense*. Boll. soc. ital. biol. sper., 30:267-269.

Morita, N., and M. Shimizu. 1963. Medicinal Resources. XXI. Flavanoids of *Cirsium* Plants in Japan. 3. Components of the leaves of *Cirsium martimum*. Yakugaku Zasshi, 83:615-618.

NAKOAKI, T., and N. MORITA, 1959. Medicinal Resources XIII. Flavanoids of *Cirsium*. 1. Components of the leaves of*C. microspecatum, C. Otayae, C. Yoshizawae, C. Japonicum,* and *C. purpuratum*. Yakugaku Zasshi, 79:1338-1340.

————. 1960. Medicinal Resources. XIV. Flavanoids of *Cirsium*. 2. Components of the leaves of *C. inundatum* and *C. kagamontanum*. Yakugaku Zasshi, 80:1296-1299.

PIATAK, D. M., and K. A. REIMANN. 1970. The Isolation of 1-Octacosanol from *Euphorbia corollata*. Phytochemistry, 9:2585-2586.

REIMANN, K. A. 1970. Phytochemical Study of *Euphorbia corollata*. M.S. thesis, Northern Illinois University.

SHELYUTA, V. L., Y. I. KOLISNICHENKO, and N. T. BUBON. 1970. Phytochemical Investigation of *Cirsium arvense*. Farm. Zh. (Kiev), 25:60-64.

SIMONSEN, J., and W. C. J. ROSS. 1957. *The Terpenes. Vol. IV*. Cambridge University Press, Cambridge, ix + 524 pp.

SIMPSON, J. C. E. 1944. The Triterpene Group. Part XI. The non-saponifable matter of *Lactucarium germanicum*, J. Chem. Soc.:283-286.

WAGNER, H., L. HORHAMMER, and W. KIRCHNER. Flavones of Compositae and Papilionactae. I. Occurrence of pectolinarin and linarin in the plant kingdom. Arch. Pharm., 293:1053-1062.

Manuscript received March 2, 1971.