in the same manner as described above to give about 5% yield of 2-thienol.

Treatment of 2-Thienyldimethylcarbinol.—A solution of 5 g. of 2-thienyldimethylcarbinol, prepared in 60% yield by adapting the method of Klages,[7] in 75 ml. of glacial acetic acid was placed in a flask and to it was added 10 ml. of 30% hydrogen peroxide and 0.5 g. of freshly fused zinc chloride. The solution immediately became bright red in color and after 24 hours it was nearly opaque. No thienol was obtained on processing the mixture.

The use of aluminum chloride or titanium tetrachloride instead of zinc chloride also was unsatisfactory.

(7) A. Klages, *Ber.*, **35**, 2633 (1902).

CHEMICAL LABORATORY
NORTHWESTERN UNIVERSITY
EVANSTON, ILLINOIS

The Distribution of Triterpenes in Rugel's Plantain[1]

BY R. C. HILTIBRAN, C. L. WADKINS AND H. J. NICHOLAS

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In connection with a study of the pentacyclic triterpenes and plant sterols now under way in this Laboratory, it appeared of interest to investigate the simultaneous occurrence of these substances and their distribution within various common plants. Zimmermann[2] has stated that only in the dandelion are triterpenes found in all parts of the plant, and Noller[3] has suggested that other plants will be found having a similar distribution. Rugel's Plantain, or *Plantago rugellii*, one of the most prolific weeds in this country, appears from the data presented here to be another such plant. The specimens employed in the present study were obtained from Missouri and Eastern Kansas and were distinguished from the almost identical species *Plantago major* by inspection of the spike.[4]

A chemical study of the alcoholic extracts of the dried and finely ground plant revealed sitosterol (as the sitosterol mixture[5]) as the only sterol present and ursolic acid and oleanolic acid as the triterpenes. The occurrence of these isomeric triterpenes in the same plant appears to be fairly unique, since only a few plants have been reported to contain ursolic and oleanolic acids together.[6-8]

The quantitative distribution of the substances studied is shown in Table I. Ursolic acid and sitosterol occur in all parts of the plant, whether young (before the appearance of seed stalks) or mature. Oleanolic acid, however, was found only in the aereal portions of the mature plant and not at all in the young plant. It appears to us that this constant association of ursolic acid and sitosterol throughout the plant suggests some close metabolic relationship.

TABLE I

RECOVERY OF URSOLIC ACID, OLEANOLIC ACID AND SITOSTEROL FROM *Plantago rugelli*

Plant part	Plant part wt., g.	Yield, g. Ursolic acid	Oleanolic acid	Sitosterol
	Young Plant			
Roots	565	0.9	...	0.8
Leaves	3819	2.3	...	3.8
	Mature Plant			
Roots	1004	0.9	...	1.2
Leaves	2625	5.0	2.0	2.9
Seed stalks	3363	0.5	1.0	0.9
Flower parts	4016	6.0	1.5	4.3

The steroid and triterpenes were isolated by procedures generally used for isolation of sapogenins,[9] and were identified by chemical methods. The absence of oleanolic acid in the young plant was substantiated by a paper chromatographic procedure which will be published at a later date.

Experimental[10]

Preparation and Fractionation of Extracts.—Large quantities of the plant were carefully washed, then dried in air. After sectioning, each part was finely ground and exhaustively extracted with hot, 95% ethanol. The individual extracts were concentrated to low volume and refluxed for two hours in approximately 2 *N* alcoholic HCl. The cooled mixture was diluted with water, extracted with 5% $NaHCO_3$ until the latter was practically colorless, and then with 5% KOH. Oleanolic acid and ursolic acid precipitated out as the K salts during the latter operation. After removal of the precipitate, extraction with 5% KOH was continued until acidification of the extract gave no precipitate. This was essential for complete separation of the carboxylated triterpenes, which tend to remain in the ether at this point.

Isolation of Sitosterol.—The neutral ether fraction was washed with distilled water and evaporated to dryness. It consisted, in each case, principally of orange pigment, a waxy hydrocarbon melting at 72-72.5° (after several crystallizations from methanol) and sitosterol in the form of the sitosterol mixture.[5] Fractionation of this crude product was best effected by chromatography on silica gel prepared according to the method of Gordon, *et al.*[11] The crude product was placed on the column with the help of a little benzene (keeping a ratio of $^1/_5$ of crude product to silica gel) and the column was washed down with low boiling petroleum ether. The first few fractions contained the orange pigment and waxy hydrocarbon. Continued elution with petroleum ether slowly removed the sitosterol; more rapid removal was effected by elution with 2% ethanol in petroleum ether. Two to four crystallizations from methanol served to bring the sitosterol to m.p. 138-140°, not depressed by admixture with an authentic sample of sitosterol mixture from soybean. It gave a characteristic color in the Liebermann-Burchard test. An acetate was prepared from a composite sample from all plant parts by refluxing with acetic anhydride in pyridine; m.p. 127°, not depressed by admixture with an authentic sample of acetate prepared from soybean sitosterol mixture.

Separation of Oleanolic and Ursolic Acids.—The KOH precipitate and KOH extracts were acidified, then extracted with ether. The latter was washed with water and distilled off, leaving a greenish, amorphous mass. The material was placed on a silica gel column ($^1/_5$ ratio of crude product to silica gel) with the help of a little warm benzene and eluted with low boiling petroleum ether until waxy solid no longer

(1) Presented before the Division of Biological Chemistry of the American Chemical Society at the autumn meeting held in Atlantic City, N. J., 1952.

(2) J. Zimmermann, *Helv. Chim. Acta*, **26**, 642 (1943).

(3) C. R. Noller, *Ann. Rev. Biochem.*, **14**, 383 (1945).

(4) J. M. Fogg, Jr., "Weeds of Lawn and Garden," Univ. of Penn. Press, Philadelphia, Pa., 1945, p. 159.

(5) L. Fieser and M. Fieser, "Natural Products Related to Phenanthrene," 3rd. Ed., Reinhold Publ. Corp., New York, N. Y., 1949, p. 285.

(6) E. J. Rowe, J. E. Orr, A. H. Uhl and L. M. Parks, *J. Am. Pharm. Assoc.*, **38**, 122 (1949).

(7) T. Bersin and A. Muller, *Helv. Chim. Acta*, **35**, 1891 (1952).

(8) C. Djerassi, *et al.*, THIS JOURNAL, **75**, 2254 (1953), in compiling a list of plant sources of oleanolic acid, were apparently unaware of our Abstract.[1]

(9) R. E. Marker, *et al.*, *ibid.*, **69**, 2167 (1947).

(10) Melting points are uncorrected and were obtained on the Fisher-Johns block.

(11) A. H. Gordon, A. J. P. Martin and R. L. M. Synge, *Biochem. J.*, **37**, 79 (1943).

came off. Continued elution with 2% ethanol in petroleum ether slowly removed the oleanolic and ursolic acids as a mixture. The isomers were readily separated in crude form at this point by boiling in methanol and filtering or decanting the partially cooled methanol solution. The supernatant or filtrate contained the oleanolic acid, since ursolic acid is only slightly soluble in hot methanol.

Isolation of Oleanolic Acid as the Acetate.—Oleanolic acid, obtained in amorphous form by evaporation of the methanol solution, was converted to the acetate for preliminary identification by boiling with acetic anhydride in pyridine. Samples of the acetate from each plant part melted sharply between 258 and 261° after several crystallizations from methanol. A composite sample from all plant parts containing oleanolic acid consisted of needles, m.p. 259–261° after two crystallizations from methanol.[12]

Anal. Calcd. for $C_{32}H_{50}O_4$: C, 77.06; H, 10.10. Found: C, 76.80; H, 9.88.

Oleanolic Acid.—Each of the oleanolic acid acetate samples was saponified with 5% alcoholic KOH to the free acid. After several crystallizations from methanol, needles melting sharply between 295 and 302° were obtained in each case. None of the samples depressed the melting point of an authentic sample of free oleanolic acid.[13] A composite sample of all of the free acid fractions, after two crystallizations from acetone, consisted of needles, m.p. 302–303°.[12] It gave a characteristic color in the Liebermann–Burchard test.

Anal. Calcd. for $C_{30}H_{48}O_3$: C, 78.89; H, 10.58. Found: C, 78.66; H, 10.38.

Oleanolic Acid Benzoate.—A composite sample of the free acid was boiled with benzoyl chloride in pyridine, yielding a benzoate, m.p. 259–261°, after several crystallizations from methanol.[14]

Isolation of Ursolic Acid as the Acetate.—Crude ursolic acid obtained as outlined above consisted of an amorphous, green powder which resisted all attempts to purify it by crystallization from various solvents. Samples from each plant part, however, were readily converted to the crystalline acetate by boiling in acetic anhydride–pyridine mixture. Each preparation melted sharply between 284 and 287° after several crystallizations from acetone or methanol. A composite sample from all plant parts, after two crystallizations from acetone, consisted of needles, m.p. 286–287°.[15]

Anal. Calcd. for $C_{32}H_{50}O_4$: C, 77.06; H, 10.10. Found: C, 77.04; H, 10.12.

Ursolic Acid.—Saponification of each of the acetate samples with 5% alcoholic KOH gave an amorphous white powder, m.p. 260–285°, which also resisted numerous attempts at crystallization from various solvents. Except in one case (mature roots) the acid was finally obtained in crystalline form by treating the saponified product according to the procedure of King, *et al.*[16] A composite sample from all plant parts, after two crystallizations from ethanol, consisted of needles, m.p. 283–285°,[15] undepressed by admixture with an authentic sample of ursolic acid.[13] It gave a characteristic color in the Liebermann–Burchard test.

Anal. Calcd. for $C_{30}H_{48}O_4$: C, 78.89; H, 10.58. Found: C, 78.62, H, 10.80.

Ursolic acid monoacetyl methyl ester, prepared according to the procedure of Rowe, *et al.*,[6] melted at 247°, in agreement with the recorded value.

Ursolic acid acetate acid chloride, prepared according to the procedure of Sando,[17] melted at 221°, in agreement with the recorded value.

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DEPARTMENT OF BIOCHEMISTRY
UNIVERSITY OF KANSAS
LAWRENCE, KANSAS

(12) A. Winterstein and G. Stein, *Z. physiol. Chem.*, **199**, 64 (1931), give m.p. 259–264° for oleanolic acid acetate and m.p. 305–308° for free oleanolic acid.

(13) Kindly supplied from the collection of Dr. C. E. Sando at the U. S. Department of Agriculture.

(14) Y. Obata, *J. Agr. Chem. Soc. Japan*, **17**, 219 (1941), gives m.p. 260–262° for oleanolic acid benzoate. See *C. A.*, **45**, 3912c (1951).

(15) Rowe, *et al.*, ref. 6, give m.p. 288–289° for ursolic acid acetate and m.p. 282–284° for free ursolic acid.

(16) N. M. King, A. Chatterjee and L. M. Parks, *J. Am. Pharm. Assoc.*, **39**, 595 (1950).

(17) C. E. Sando, *J. Biol. Chem.*, **90**, 477 (1931).

The Reaction of Ethyl Trichloroacetate with Aromatic Grignard Compounds

BY A. KALUSZYNER AND S. REUTER

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Henry[1] has described the reaction between methylmagnesium bromide and ethyl trichloroacetate (I) as leading to the expected trichloro-*t*-butyl alcohol, without indicating the yield. Experiments with aromatic Grignard compounds, undertaken in an attempt to synthesize substances of the general formula CCl_3CAr_2OH, have led to unexpected results.

The reaction of I with phenylmagnesium bromide (4 moles per mole ester) did not yield the chlorinated tertiary alcohol in isolable quantities. Apart from about 0.4 mole of biphenyl, a yield of 1 mole of chlorobenzene was obtained, identified by boiling point, density and refractive index. In the aqueous layer obtained in the decomposition of the reaction product, one third of the chlorine of the ester I was recovered in form of chloride ion. Phenyllithium reacts with I in the same manner.

The analogous reaction with *p*-tolylmagnesium bromide gave 0.6 mole of *p*-chlorotoluene, 0.8 mole of 4,4′-dimethylbiphenyl and in the aqueous layer, 80% of the chlorine of I as chloride ion. The reaction with *p*-chlorophenylmagnesium bromide yielded 0.2 mole of *p*-dichlorobenzene, 0.8 mole of 4,4′-dichlorobiphenyl and 72% of the chlorine atoms of I in ionic form.

It appears, therefore, that ethyl trichloroacetate reacts with arylmagnesium bromides according to the following equation to give the corresponding aryl chloride

$$ROOC{\cdot}CCl_3 + ArMg\,Br \longrightarrow ArCl + (ROOC{\cdot}CCl_2)^-MgBr^+ \quad (1)$$

In the case of phenylmagnesium bromide, all of the trichloroacetate reacts in that manner, in that of *p*-tolylmagnesium and of *p*-chlorophenylmagnesium bromide, only 60 and 10%, respectively.

Replacement of MgX in Grignard compounds by chlorine has been known to occur with substances containing "positive chlorine," such as aryliodonium chloride,[2] ethyl hypochlorite,[3] N-chloropiperidine[4] and benzene sulfochloride.[5] One has, therefore, to conclude that in ethyl trichloroacetate (I), the accumulation of chlorine atoms gives them an electropositive character.

For the formation of chloride ion and the biaryl compound in the reaction between an arylmagnesium bromide and ethyl trichloroacetate, a number of possibilities exist. In this respect, it is re-

(1) L. Henry, *Bull. soc. chim. Belg.*, **20**, 152 (1906); *Chem. Zentr.*, **77**, 1178 (1906).

(2) H. Hepworth, *J. Chem. Soc.*, **119**, 1244 (1921).

(3) N. N. Melnikow, *Chem. Zentr.*, **107**, II, 2896 (1936).

(4) R. I. W. Le Fèvre and P. I. Markham, *J. Chem. Soc.*, 703 (1934).

(5) R. I. W. Le Fèvre, *J. Chem. Soc.*, 1245 (1932); *cf.* H. Gilman and R. E. Fothergill, THIS JOURNAL, **51**, 3501 (1929).