acids obtained by GLC is not clear. Reactions may have occurred between the epoxides and any of the reactive constituents not ordinarily found together with them under conditions of the GLC analysis. Losses occurring from decomposition or alteration of epoxy acids on a number of stationary phases have been investigated by Herb and co-workers (26).

Evidence for the presence of small amounts of a dihydroxy acid is shown in sample 3 of Figure 4. Formation of unusual oxygenated compounds, probably methyl ethers of the hydroxy acids, is suggested by TLC of esters prepared with methanolic sulfuric acid (sample 4, Fig. 3). These oxygenated materials are undergoing further study.

ACKNOWLEDGMENTS

GLC analyses by J. W. Hagemann; *Helichrysum bracteatum* seeds supplied by Quentin Jones, New Crops Research Branch, ARS, USDA.

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[Received September 10, 1964—Accepted January 18, 1965]

Search for New Industrial Oils. XII. Fifty-eight Euphorbiaceae Oils, Including One Rich in Vernolic Acid[1]

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Abstract

Seed oil of *Euphorbia lagascae* Spreng. contains 57% of *cis*-12,13-epoxy-*cis*-9-octadecenoic (vernolic) acid. The amt of trivernolin in the glycerides of this species indicates random or restricted random distribution of the vernolic acid.

Seed from 57 additional species in the Euphorbiaceae were analyzed for oil and protein contents and also for fatty acid composition of the oils. Iodine values (I.V.) of the oils ranged from 87–221. Among these oils, samples were encountered with as much as 76% linolenic, 77% linoleic or 84% oleic acid.

Introduction

OF THE GLYCERIDE OILS now traded in quantity, two of the more unusual ones are from plants in the Euphorbiaceae (spurge family). Castor oil containing ricinoleic acid is obtained from *Ricinus communis* L., and tung oil containing eleostearic acid from several species of *Aleurites*. Literature reports of other unusual oils in the family include kamala oil from *Mallotus philippinenis* Muell. Arg. containing hydroxyeleostearic acid; croton oil from *Croton tiglium* L. possessing violent purgative properties; oil containing epoxy acids from *Cephalocroton* (1); and oils containing conjugated unsaturation from *Ricinodendron, Sapium,* and *Garcia*. Most of the oils from approx 65 species of Euphorbiaceae reported by Hilditch (10), Eckey (6), and in more recent literature contain only the common fatty acids in widely varying proportions.

In our continuing survey of seed oils, 58 species of Euphorbiaceae have been analyzed; 11 of these, including six in our earlier papers (3,4), have been reported in prior literature but without gas-liquid chromatographic (GLC) analyses. One species of the 58, *Euphorbia lagascae* Spreng., is unique in its high content of epoxyoleic acid.

The Euphorbiaceae include some 280 genera and 8,000 species (2), predominantly tropical but also widely distributed in temperature regions. The largest genera are *Euphorbia* (ca. 1,000 species), *Croton* (ca. 500–600 species), and *Phyllanthus* (ca. 400 species). Plant types range from herbs to trees and include vine- and cactus-like forms. Useful commercial products other than oils obtained from the family include rubber (*Hevea brasiliensis* Muell. Arg.), candelilla wax (*Euphorbia antisyphilitica* Zucc.), and cassava (*Manihot esculenta* Cranz.). Many species are grown domestically as ornamentals. The samples analyzed represent two of the four subfamilies and 10 of the 11 tribes within these subfamilies.

Materials and Methods

Collection, preparation, analysis of seed, and GLC analysis of the fatty acids were accomplished as previously described (5,15,18). Seed of *Euphorbia lagascae* was collected from wild plants in Spain under Public Law 480 funds. Methyl esters were prepared by acid-catalyzed methanolysis except for the *E. lagascae* preparation that was catalyzed by sodium methoxide (16).

[1] Presented at the AOCS in New Orleans, 1964.
[2] A laboratory of the No. Utiliz. Res. & Dev. Div., ARS, USDA.
[3] ARS, USDA.

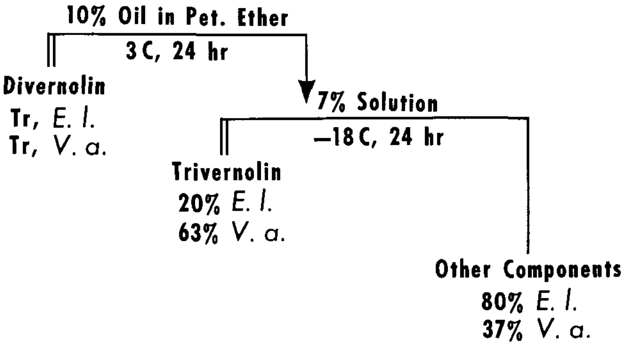

FIG. 1. Low-temp fractionation of *Euphorbia lagascae* (E.l.) and *Vernonia anthelmintica* (V.a.) oils. (Tr = Trace).

Structure of the epoxy acid in *E. lagascae* oil was established by procedures essentially like those described by Gunstone (8). The oil was treated with boiling glacial acetic acid, saponified with 1 *N* KOH/EtOH, acidified strongly with HCl, and then extracted with ethyl ether. In this process the expoxy acid was converted to a dihydroxy acid which in turn was isolated by partitioning between 90% methanol and petroleum ether, recovered from the methanol phase by evaporation and recrystallized from acetone. A portion of the isolated dihydroxy acid was oxidized with permanganate-periodate reagent, and another portion was hydrogenated to eliminate unsaturation before oxidation. Oxidation products were analyzed as free acids and as methyl esters by GLC on a polar (LAC-2-R446) column and a nonpolar (Apiezon L) column to identify the oxidation products.

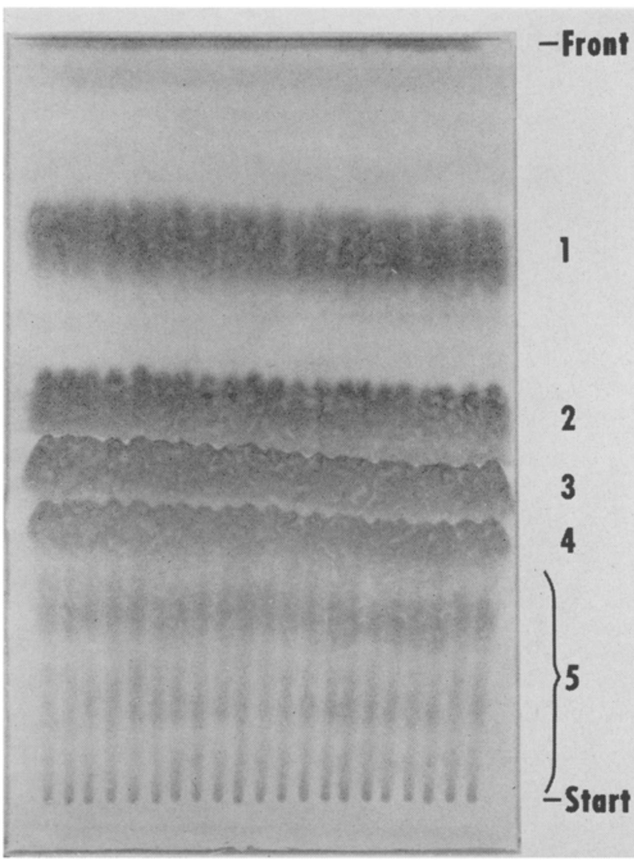

FIG. 2. Preparative TLC of *Euphorbia lagascae* oil. 1 μl spots. Solvent travel, 17 cm. Area 1, nonoxygenated triglycerides; Area 2, 31% vernolate in triglyceride; Area 3, 64% vernolate in triglyceride; Area 4, trivernolin (98% vernolate); and Area 5, unknown.

For the identification of the octadecenoic acid in selected oils, the monoenoic fraction was collected from the mixed methyl esters either by thin-layer chromatography (TLC) on a 1-mm film of Silica Gel G (19) impregnated with 30% silver nitrate with benzene as the developing solvent, or by trapping the 18:1 component [equivalent chain length (ECL) 18.3] from a 20 ft x 3/8 in copper GLC column packed with 20% LAC-2-R446 on 60/80 mesh Celite. The recovered ester was oxidatively cleaved and analyzed as above.

Oil for the triglyceride structure studies was extracted from the whole seed by grinding in a Waring Blendor in cold petroleum ether (5C) or from autoclaved seed (15 psi for 30 min) by grinding with the same solvent at room temp (11,12). Trivernolin was isolated from the oil of *E. lagascae* by: A) a modification of the method used by Krewson et al. (11) to isolate trivernolin from *Vernonia anthelmintica* Willd. (Fig. 1), or B) preparative TLC. For comparison, method A was also applied to *V. anthelmintica* oil.

The preparative TLC plates were made with ether-washed Silica Gel G (500 μ thickness). The plates were developed with petroleum ether (40–50C) : ethyl ether : glacial acetic acid (80:20:1 v/v), air dried and redeveloped with the same reagents in a ratio of 90:10:1. The trivernolin area and the remainder of the sample were scraped separately from the plate, and fractions were recovered from the support by extraction with ether. In method B the areas were made visible by spraying locator strips on both sides of the plate with a 0.2% ethanolic solution of 2′,7′-dichlorofluorescein. The locator strips were discarded.

In both methods fractions isolated from the oil of *E. lagascae* were analyzed by TLC (films of 250 μ thickness) and by GLC. Spots on the TLC plates were located with iodine vapor and recorded with black Diazo paper (7).

Hydrogen bromide titrations for epoxides were carried out at 55C for all samples and in addition at 3C (9) for *E. lagascae.*

Results and Discussion

The initial analyses (Table I) of all but two of the samples in this group show no significant amt of unusual components. GLC confirms that these oils are composed of common fatty acids but in widely varying proportion. Oil from *Sapium sebiferum* seed kernels absorbs UV light at 266 mμ, and GLC shows a component (5%) presumed to be the previously reported 2,4-decadienoic acid (6). Other unidentified short-chain components total 3% by GLC. Oil from *E. lagascae* seed contains HBr-absorbing material equivalent to 58% of C_{18}-epoxy monoenoic acid. IR absorption at 11.85 μ and 12.17 μ indicate that the reactive material is an epoxide. Following Gunstone's procedure (8) for identification of the epoxy acid we obtained a crystalline dihydroxy acid melting at 48–50C (lit mp for product from vernolic acid, 49–51.5C). Products of oxidation of the unsaturated dihydroxy acid were shown by GLC to be 35% hexanoic and 60% nonanedioic (azelaic) acids. Hydrogenation of the unsaturated acid gave a product which had no melting point depression on admixture with authentic *threo*-12,13-dihydroxyoctadecanoic acid. Oxidation of the saturated acid gave 34% hexanoic and 63% dodecanedioic acids. The original component of the oil from which the dihydroxy acid was prepared is therefore *cis*-12,13-epoxy-*cis*-9-octadecenoic (vernolic) acid.

Fractionation of oil from *E. lagascae* provides a trivernolin fraction (Fig. 1) of purity comparable to

TABLE I

Analytical Data on Euphorbiaceae Seeds and Oils

Source	Seed analysis				Oil properties		Composition of methyl esters, % by GLC						
	Wt / 1,000, g	Oil content, % D.B.	Protein content, N × 6.25, % D.B.	Component analyzed[a]	Iodine value	Refractive index n_D^{40}	16:0	18:0	18:1	18:2	18:3	20:1	Other components
Phyllanthoideae													
Phyllantheae													
Breynia rhamonides Muell.	1.1	27	16	S		.....	15	11	14	21	33	0.8	5
Phyllanthus abnormis Baillon	17	30	14	S	160	1.4705	9	3	27	23	37	0.5	1
Reverchonia arenaria A. Gray	31	28	11	S + P	161	1.4710	9	3	26	21	40	0.6	0.4
Bridelieae													
Bridelia stipularis Blume	21	18	15	S	148	1.4705	11	10	20	26	32	0.5	1
Daphniphylleae													
Daphniphyllum humile Maxim.	50	35	15	S	104	1.4644	9	4	58	28	0.2		0.4
Daphniphyllum macropodium Miq.	41	37	18	S − SC	97	1.4646	12	4	55	28	0.4	0.2	0.3
Crotonoideae													
Crotoneae													
Croton capitatus Michx.	10	15	21	S	166	1.4726	7	3	13	41	35	0.4	Trace
Croton corymbulosus Engelm.	6.6	24	23	S	176	1.4730	6	3	12	37	39	1	1
Croton fragilis H.B.K.	14	25	27	S	142	1.4690	8	4	9	70	6	1	2
Croton gracilis H.B.K.	30	29	30	S	176	1.4738	6	2	9	50	30	1	2
Croton texensis Muell. Arg.	3.2	26	25	S	169	1.4740	4	2	10	49	30	4	1
Eremocarpus setigerus (Hook.) Benth.	3.2	28	28	S + P	140	1.4685	6	3	12	73	1	0.3	4
Acalypheae													
Chrozophora hierosolymitana Spreng.	17	28	22	S + P	144	1.4694	5	5	12	77	0.8	Trace	0.5
Chrozophora tinctoria A. Juss.	13	56	35	S − SC	142	1.4684	6	5	12	75	1	0.2	0.3
Mercurialis annua L.	1.5	37	19	S	211	1.4763	5	7	8	11	68	.	0.5
Tragia incana Baill.	31	28	25	S + P	184	1.4747	6	3	14	33	44	0.5	0.4
Jatropheae													
Cnidoscolus angustidens Torr.	51	44	42	S	119	1.4645	19	6	13	59	0.8	0.3	1
Cnidoscolus elasticus Lundell	787	26	13	S	119	1.4665	17	6	16	59	1	0.3	0.2
Cnidoscolus tepiquensis McVaugh	1105	20	13	S	113	1.4656	13	8	23	54	0.9	0.4	0.6
Jatropha cordata (Orteg.) Muell. Arg.	245	39	16	S	120	1.4657	11	8	23	57	0.7	Trace	1
Jatropha curcas L.	430	31	19	S	124	1.4670	8	7	23	59	0.7	0.3	1
Jatropha hestata Jacq.	40	14[b]	...	S	....	....	8	3	13	66	8	0.3	2
Jatropha macrorhiza Benth.	101	53	36	S	133	1.4673	8	4	18	68	0.8	0.3	1
Jatropha spathulata Muell. Arg.	127	58	26	S − SC	119	1.4659	12	5	28	53	0.3	....	1
Adrianeae													
Manihot isoloba Standley	243	32	21	S	121	1.4661	13	4	23	57	0.5	0.8	1
Manihot tweedieana Muell. Arg.	86	24	15	S	138	1.4685	8	3	22	62	4	0.3	0.5
Cluytiae													
Cluytia affinis Sond.	7.0	42	23	S	182	1.4730	8	3	15	22	50	2	0.1
Hippomaneae													
Sapium haematospermum Muell. Arg.	40	25	13	S	181	1.4717	10	2	14	19	54	0.1	2
Sapium montevidense Klotzsch	28	13	18	S + P	176	1.4748	12	2	14	16	53	....	2
Sapium sebiferum (L.) Roxb.	[150	45	10	S + P[c]	184	1.4784	5	2	14	26	46	....	8[d]
	[........	....[e]	....	....	......	....	70	1	28	0.9	....	....	Trace
Euphorbieae													
Euphorbia amygdaloides L.	1.8	40	22	S	201	1.4760	6	0.9	11	18	63	0.4	0.3
Euphorbia anacampseros Boiss.	5.5	30	16	S	210	1.4766	5	1	11	13	69	0.6	0.1
Euphorbia bicolor Engelm. & Gray	17	27	23	S	195	1.4740	6	2	14	16	58	3	0.8
Euphorbia clavigera N.E. Br.	2.4	37	20	S	199	1.4770	6	2	13	17	61	0.6	0.4
Euphorbia cornigera Boiss.	5.6	37	30	S	199	1.4750	6	1	11	24	58	0.2	0.6
Euphorbia cuphosperma Boiss.	0.4	7	8	S	....	....	12	5	14	15	51	0.3	3
Euphorbia cybirensis Boiss.	3.1	42	24	S	204	1.4756	6	1	11	14	66	0.7	0.3
Euphorbia dracunculoides Lamb.	36	36	14	S	191	1.4753	7	2	13	24	53	0.1	0.4
Euphorbia eriophora Boiss.	0.8	21	15	S	...	....	8	2	13	23	51	0.5	2
Euphorbia falcata L.	3.6	47	14	S	182	1.4737	7	2	18	23	49	0.1	0.3
Euphorbia geniculata Orteg.	7.2	23	27	S	192	1.4760	9	4	8	21	58	0.2	0.3
Euphorbia heterophylla L.	6.8	38	26	S	198	1.4742	6	4	9	22	59	0.3	Trace
Euphorbia kotschyana Fenzl	2.6	36	39	S − SC	186	1.4747	8	2	17	17	54	1	0.8
Euphorbia lagascae Spreng.	7.4	42	26	S	88	1.4676	4	2	20	12	0.5	0.8	60[f]
Euphorbia lathyrus L.	36	48	15	S	87	1.4645	7	2	84	3	3	1	....
Euphorbia marginata Pursh	9.4	18	16	S + P	202	1.4769	6	2	12	14	63	0.5	2
Euphorbia mauritanica L.	7.9	45	18	S	195	1.4766	8	2	8	19	62	0.2	0.4
Euphorbia medicaginea Boiss.	1.3	41	18	S	201	1.4779	6	1	14	10	66	0.4	2
Euphorbia myrsinites L.	2.1	32	27	S + P	217	1.4780	4	1	11	11	72	0.3	Trace
Euphorbia paralias L.	4.3	41	22	S	183	1.4742	8	2	19	15	55	0.6	0.4
Euphorbia parryi Engelm.	0.5	44	22	S + P	221	1.4780	5	2	6	12	76	....	Trace
Euphorbia salicifolia Host	23	31	24	S	188	1.4735	6	0.9	14	23	55	0.9	0.7
Euphorbia segetalis L. var. *littoralis*	2.5	40	23	S	192	1.4775	8	1	16	13	60	0.2	1
Euphorbia serrata L.	7.6	42	21	S	198	1.4747	6	1	12	26	55	0.4	0.2
Euphorbia terracina L.	2.6	32	18	S	197	1.4751	7	1	12	21	55	0.6	4
Euphorbia thamnoides Boiss.	3.7	34	26	S	205	1.4760	6	1	9	17	66	0.3	0.4
Euphorbia tinctoria Boiss. & Huet	2.4	24	32	S	214	1.4784	5	0.9	9	13	72	0.4	0.4
Pedilanthus macrocarpus Benth.	96	62	16	S − SC	139	1.4708	12	4	23	38	22	0.1	0.7

[a] S = Seed; S + P = seed plus pericarp; S − SC = seed minus seed coat.
[b] "As is" basis.
[c] Iodine value, refractive index and methyl ester composition on kernel oil only.
[d] 5% 2,4-Decadienoate.
[e] Washing the unground seed with hot benzene provided 24% of pericarp oil for analysis.
[f] 57% Vernolate.

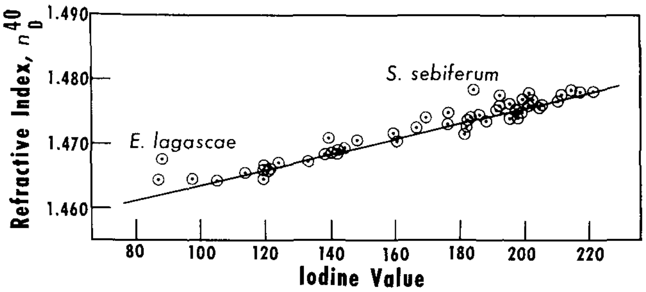

FIG. 3. I.V./refractive index relationship among oils of the Euphorbiaceae.

that obtained from vernonia oil but representing only 20% of the oil instead of 63% as with *Vernonia*. As an independent measure of trivernolin, TLC of 29.1 mg of *E. lagascae* oil on Silica Gel G (Fig. 2) gave a total recovery of 29.7 mg, of which 5.5 mg (18.5% of the original oil) migrated at the same rate as authentic trivernolin. The methyl esters of this fraction were shown by GLC to be 98% methyl vernolate. The percentage of trivernolin in *E. lagascae* oil is in excellent agreement with that expected from random or restricted random distribution (18.8 and 18.6%, respectively). It is in sharp contrast to the results for vernonia oil in which essentially all of the vernolic acid occurs as trivernolin (11).

Five percent of the *E. lagascae* seed consists of an easily detachable caruncle which contains 24% oil. The fatty acid content of this seed component is mainly 18:0 and 18:1, 12 and 74%, respectively. In contrast to the high percentage of vernolic acid present in the whole *E. lagascae* oil, the oil in the carnucle had only 3.6% hydrogen bromide equivalent when calculated as epoxyoleic acid.

The GLC analysis of the oxidation products of the monoenoic acids of *Cnidoscolus tepiquensis, E. lathyrus,* and *E. lagascae* showed two major components (total greater than 95%), azelaic acid and pelargonic acids. Since the IR spectra of these oils gave no indication of *trans* unsaturation, in each instance the 18:1 acid is oleic.

The variability of all these oils is strikingly indicated by plot of refractive index against I.V. (Fig. 3). Except for the two oils containing unusual components all fall close to the regression line reported in Part 1 of this series (5). Both the lowest and highest I.V. are from the genus *Euphorbia: E. lathyrus,* I.V. 87, long known to contain ca. 85% oleic acid (6,10), and *E. parryi,* I.V. 221, shown in this study to contain 76% octadecatrienoic acid, probably linolenic. Those oils with I.V. below 105 are rich in octadecenoic acid (55–85%); those between 110 and ca. 144, in octadecadienoic acid (50–77%); and those from ca. 181–221, in octadecatrienoic acid (50–76%).

Table I is arranged by subfamilies and tribes to reveal similarities within groups. The eight members of the Jatropheae bear large seed (40–1,105 g/1,000); their oils have intermediate I.V. (113–133) and contain "linoleic" acid as the major component (53–68%). In the tribe Euphorbieae, the C_{18}-trienoic acid is the major component except in oils of *E. lathyrus, E. lagascae* and *Pedilanthus macrocarpus.* Other generalizations are not so obvious, either because of a variable composition or perhaps because of too few representatives within a given group.

Many of the oils contain small amt of various acids reported in Table I as "other components." These include up to 3% of 14:0 in 50 samples, up to 1% of 16:1 in 8 samples, up to 1% of 20:0 in 27 samples, up to 4% of 20:1 in 59 samples, a trace of 22:0 in 2 samples and up to 3% of 22:1. Several oils contain minor amt of unknown components, including apparent 15:0, 15:1, 17:0 and 17:1.

Many species of the Euphorbiaceae produce seed containing toxic, purgative or allergenic principles. No tests for such components were included in the present study. Layton et al. (13,14) showed that individuals allergic to castor bean also react to preparations from *Cnidoscolus texanus* (Muell. Arg.) Small, *Euphorbia esula* L. and *Poinsettia pulcherrima* R. Grah. (*Euphorbia pulcherrima* Willd.) and suggested that such sensitivity might extend to the whole family Euphorbiaceae.

TABLE II

Grams of Amino Acid/16 Grams Nitrogen in *Euphorbia lagascae*

Lysine	4.0	Arginine	12.5
Methionine	2.6	Glycine	4.3
Isoleucine	4.4	Alanine	4.6
Leucine	6.4	Aspartic acid	11.6
Phenylalanine	5.3	Glutamic acid	16.2
Tyrosine	2.6	Hydroxyproline	0.3
Threonine	3.6	Proline	4.0
Histidine	2.4	Serine	4.5
		Valine	5.6

The amino acid composition of *E. lagascae* seed protein (Table II) is similar to that in the five Euphorbiaceae species previously reported from the Northern Laboratory (17,18). The lysine level lies between that of legumes and cereals; the methionine level is comparable to that of the cereals but distinctively above that of the legumes.

Studies of seed yields, methods of production and areas of adaptability of those species producing the common fatty acids have no particular urgency until a need for this information becomes more evident. The unusual oil of *E. lagascae* justifies immediate study of this species. This plant is a small (1–1.5 ft), coarse annual which flowers in early spring and fruits in late spring in the warm Mediterranean region where it is native. It is often found in fallow land or other areas disturbed by man; consequently it might be readily adaptable to conditions of cultivation.

ACKNOWLEDGMENTS

Trivernolin, 1,3-divernolin, vernolic acid, and *Vernonia anthelmintica* seed oil provided by C. F. Krewson, E. Reg. Res. Laboratory; many of the GLC analysis by J. W. Hagemann; amino acid analysis by J. E. Peters.

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[Received October 31, 1964—Accepted November 24, 1964]